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Review article: vitamin D and inflammatory bowel disease – established concepts and future directions

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SUMMARY

Background
Understanding of the role of vitamin D in health and disease has increased markedly in the past decade, with its involvement extending well beyond traditional roles in calcium and phosphate homeostasis and musculoskeletal health. This conceptual expansion has been underpinned by identification and exploration of components of this axis including vitamin D-binding protein, key enzymes and receptors in multiple cell types, and a greater recognition of nonclassical autocrine and paracrine effects. Its influence in IBD remains uncertain.

Aim
To review the role of vitamin D in bone health, immune regulation and cancer prevention in IBD, and to outline practical issues and limitations of its use.

Methods
An extensive online literature review including PubMed and Medline.

Results
In patients with IBD, the vitamin D axis provides an important and often underutilised pathway to preserving bone health. Furthermore, an exciting body of clinical and basic science research demonstrates that these pathways may have an integral part to play in regulation of the immune response in IBD, through effects on the intestinal barrier, antigen presenting cells and adaptive T cells. The possibility of chemoprevention requires further study. The optimal target level of 25-hydroxy vitamin D in patients with IBD is currently uncertain, as is the best therapeutic modality.

Conclusions
Study of vitamin D pathways may result in the development of relatively inexpensive therapeutic options to optimise patient outcomes. Further prospective clinical research is required to address efficacy and long-term safety.

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INTRODUCTION

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is a group of debilitating conditions associated with a dysregulated mucosal immune response to intestinal microorganisms in a genetically susceptible host. These conditions affect up to 0.5% of the population in developed countries and an increasing proportion in developing nations.[1–3] Apart from local intestinal complications, patients with IBD are at increased risk of systemic immune-related phenomena, nutritional deficiencies and bone disease, particularly osteoporosis.

Vitamin D is well recognised for its involvement in calcium homeostasis and musculoskeletal health. In addition, our understanding of its role in a variety of other systems and pathologies has rapidly expanded in the past decade. A wealth of *in vitro* and emerging clinical data suggests it may play a role in effective immune response, cardiovascular and renal physiology and protection against some cancers. This review will address physiological aspects of vitamin D and their relevance to IBD.

VITAMIN D SYNTHESIS, METABOLISM AND CELLULAR EFFECTS

Synthesis & absorption

Vitamin D exists in two main forms, vitamin D3 (VD3, cholecalciferol) and vitamin D2 (VD2, ergocalciferol), differing in their side chain structure. In humans, the majority of VD3 is produced in the skin from exposure to sunlight (Figure 1), with a small proportion obtained from animal sources such as oily fish and egg yolk.[4] VD2 is predominantly obtained from plant sources. Supplements of both VD2 (produced from irradiation of the yeast sterol ergosterol) and VD3 are commercially available.[5] Commonly, vitamin D refers collectively to VD2 and VD3.

Human skin-derived VD3 is produced from 7-dehydroxycholesterol upon exposure to ultraviolet B radiation (UVB, wavelength 290–315 nm).[4] As a fat-soluble vitamin, dietary vitamin D is incorporated into chylomicrons and transported via lymphatics into the venous circulation. Some of the dietary vitamin D is extracted by adipose tissue and muscle, but the remainder and most of the endogenously synthesised vitamin D is transported to the liver. Here, it is metabolised by the cytochrome P450 enzymes vitamin D 25-hydroxylases (microsomal CYP2R1 and mitochondrial CYP27A1) to 25-hydroxy vitamin D (25(OH)D).[6] In classical calcium-related responses, another cytochrome P450 enzyme, 1α-hydroxylase (CYP27B1), converts 25(OH)D to the biologically active form of vitamin D, 1,25-hydroxy vitamin D ($1,25(OH)_2D$) in the proximal tubule of the kidneys.[7, 8]

Storage, circulation and excretion

Previously believed to be biologically inert at physiological levels, 25(OH)D is the major storage and circulating form of vitamin D and frequently measured as an index of vitamin D status. In the human body, the highest concentration of 25(OH)D is noted in the plasma (usually measured in the serum as 20–150 nmol/L or 8–60 ng/mL), but the largest pool of 25(OH)D is in adipose tissue and muscle.[9] Hence, although a circulating half-life of 25(OH)D is approximately 10–15 days,[6, 10] release from tissue stores effectively results in a half-life *in vivo* of 2–3 months.[11]

Renally produced $1,25(OH)_2D$ circulates in the blood at levels in the picomolar range, about one thousandth those of 25(OH)D. Contrasting with the relative lack of regulation of 25-hydroxylation, 1α-hydroxylation is under control by serum parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) in response to serum calcium and phosphate and represents the rate-limiting step in the synthetic pathway.

Metabolites in the vitamin D pathway are transported in the circulation predominantly (about 85–90%) bound to vitamin D-binding protein (DBP, also known as group-specific component globulin, Gc-globulin) and albumin (about 10–15%), with <1% in the free form.[12] DBP is a liver-derived, 58 kDa glycosylated α-globulin structurally similar to albumin,[13] which circulates at concentrations of 0.6–11 μmol/L.[12] The affinity of DBP for 25(OH)D is 5×10^8 mol/L, about an order of magnitude greater than that for vitamin D (1×10^5 to 1×10^7 mol/L) or $1,25(OH)_2D$ (2×10^7 mol/L).[14, 15] This difference in affinity partly accounts for the shorter plasma half-life of vitamin D (~4–6 h) and $1,25(OH)_2D$ (~4–20 h).[4, 6]

Tight regulation of 1α-hydroxylation and the short half-life mean than the serum concentration of $1,25(OH)_2D$ is not an accurate measurement of total body vitamin D status, and measurement is of most use in altered states of 1α-hydroxylation such as chronic kidney disease (reduced) or granulomatous disease (increased).

The catabolic enzyme 24-hydroxylase (CYP24A1) is responsible for the conversion of both 25(OH)D and $1,25(OH)_2D$ to the inactive metabolites, $24,25(OH)_2D$ and 25(OH)D-26,23-lactone,[6] and via a multistep pathway to the water soluble calcitroic acid (1α-hydroxy-23

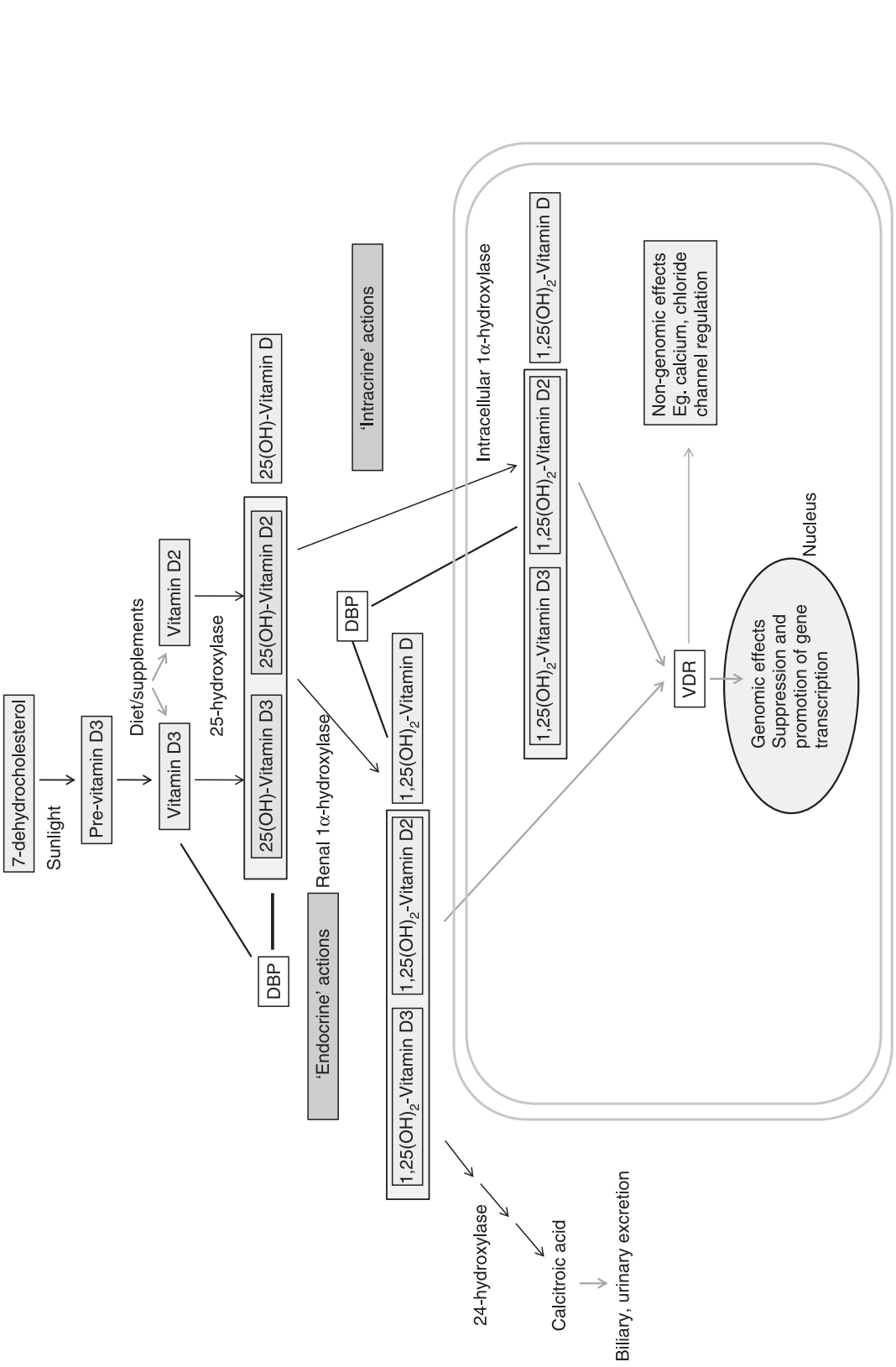

Figure 1 | Synthesis, metabolism and cellular effect of vitamin D. The classical pathway involves renal conversion of 25(OH) vitamin D to $1,25(OH)_2$ vitamin D, which circulates with subsequent 'endocrine' action on bone metabolism. A novel pathway involves uptake of 25(OH) vitamin D, either free or bound to DBP, followed by intracellular 1α hydroxylation in nonrenal cells including monocytes and colonic epithelial cells to $1,25(OH)_2$ vitamin D leading to 'intracrine' actions localised to those cells. The latter may be important in many of the extra-skeletal effects of vitamin D, particularly in cells involved in immune responses.

carboxy-24,25,26,27-tetranorvitamin D3), which undergoes urinary and biliary excretion.[16, 17]

The vitamin D-binding protein

Vitamin D-binding protein has been shown to regulate the effect of vitamin D metabolites in target organs. As a reservoir with vastly greater circulating levels than that of vitamin D metabolites, DBP may control availability in tissues, allowing only the small free fraction of vitamin D metabolites to passively enter cells through diffusion across cell membranes.[18] In a small series of 49 young adults, bone mineral density (BMD) was positively correlated with only free and bioavailable 25(OH)D, not total 25(OH)D.[12] Furthermore, BMD was negatively correlated with DBP concentrations ($r = -0.296$).[12] Second, DBP itself actively facilitates the uptake of bound 25(OH)D into renal tubular cells via the membrane glycoproteins, *megalin and cubilin*. The megalin-DBP-25(OH)D complex is internalised via endocytosis, in co-operation with cubulin, and presented to mitochondrial 1α-hydroxylase.[19] A similar process has been described in mammary cells [20] and osteoblasts.[21, 22] Third, DBP may modulate intracellular actions of 25(OH)D and 1,25$(OH)_2$D. Interestingly, DBP has been shown to attenuate monocyte response *in vitro* to 25(OH)D and 1,25$(OH)_2$D, seemingly independent of megalin.[23] Loss of DBP in the urine in proteinuric diseases has been suggested to be a cause for vitamin D deficiency associated with diabetes.[24]

The gene for DBP has three dominant single nucleotide polymorphisms (SNPs), which correlate with protein products (GC-1s, GC-1f and GC-2) separated by single amino acid substitutions.[25] Functionally, GC-1s has twice and GC-1f has four times the binding affinity for 25(OH)D than GC-2.[26] In individuals, these SNPs result in six diplotypes (1f-1f, 1f-1s, 1s-1s (collectively GC-1-1), 1f-2, 1s-2 (collectively GC-1-2) and 2-2 (GC-2-2)). Subsequently, total 25(OH)D levels are lowest in subjects with GC-2-2, intermediate in those with GC-1-2 and highest in those with GC-1-1.[27–30] The functional significance of this polymorphism is not yet completely understood, but may cause very different circulating free and hence intracellular levels of 25(OH)D3.[31] This means that it is difficult to ascertain deficiency or repletion without knowledge of free levels and DBP genotype.

Apart from binding vitamin D metabolites, DBP also binds with high affinity globular (G)-actin via a domain near its C-terminus, preventing the formation of filamentous (F)-actin, which plays a role in vascular occlusion following cellular damage.[18, 25] Subsequently, any condition which results in cell death, and hence the release of large amounts of G-actin, may reduce free plasma DBP.[18, 32] This has been noted in systemic sepsis.[32] However, given DBP is produced in the liver with close homology to albumin, its synthesis may also be reduced in any condition of physiological stress.[33] Other described functions of DBP include enhancement of the neutrophil chemotactic effect of complement 5a,[34] and deglycosylated DBP serves as a macrophage-activating factor.[35]

Several studies have investigated the association of risk of specific diseases with DBP genotype, but no consistent relationship has yet been established.[36–38]

The Vitamin D Receptor (VDR)

The VDR was first recognised by Haussler and colleagues in 1969,[39, 40] and its structure subsequently described in 1988.[41] Over the past three decades, increasing characterisation of the role of the VDR and its presence in multiple tissues has resulted in a vast expanse in our knowledge of the pleiotropic nature of the vitamin D axis in human physiology.

Most currently described cellular actions of vitamin D occur via genomic regulation. 1,25$(OH)_2$D, on entry into cells or via intracellular conversion from 25(OH)D, binds to nuclear VDR. The VDR-ligand complex subsequently forms a heterodimer with retinoid-X receptor and binds vitamin D responsive elements located predominantly in the promoter regions of target genes.[16] In combination with transcription factors and co-regulatory proteins, this complex has been shown to promote or suppress the transcription of a wide range of genes. Through recent advances using chromatin precipitation with massively parallel sequencing (ChIP-seq) in lymphoblastoid cell lines, 623 genomic regions were shown to be occupied by VDR in the basal state, increasing to 2776 regions on stimulation with calcitriol, with significant changes in expression in 229 identifiable genes.[42]

In addition to regulation of gene transcription, VDR also participates in nontranscriptional rapid cellular responses by translocating to the plasma membranes within seconds or minutes (compared with hours to days required for genomic regulation).[43] Examples of these actions include voltage-gated calcium and chloride channel regulation in osteoblasts, skeletal muscle cell calcium entry, contractility and myogenesis, calcium uptake by intestinal epithelial cells and insulin secretion by pancreatic islet beta cells.[43, 44]

Inactivating mutations in the VDR gene result in hereditary vitamin D-resistant rickets, a condition

manifested by rickets, hypocalcaemia, hypophosphatemia and alopecia.[45] More subtle mutations or polymorphisms in the VDR gene have been described. The more widely studied polymorphisms include *Taq*I, *Bsm*I, *Apa*I, *Tru*9I and *Eco*RV between exons 8 and 9, *Fok*I in exon 2 and *Cdx*2 near exon 2.[46] These changes may affect gene promotion, RNA transcription efficiency or protein structure.[46] For example, a *Fok*I polymorphism resulting from a T to C change shifts the start codon, and hence results in a protein that is truncated by three amino acids. This shorter protein has greater transcriptional activity due to increased binding affinity to transcription factor IIb.[47] Intense research is actively underway to find associations of various polymorphisms with diseases, but much data to date have been conflicting.[46]

CLASSICAL ROLE OF VITAMIN D: CALCIUM HOMEOSTASIS AND MUSCULOSKELETAL HEALTH

The predominant actions of vitamin D are to increase serum calcium and phosphate, and to promote bone mineralisation. Parathyroid hormone is the main stimulus to synthesis of the active metabolite $1,25(OH)_2D$. Hypophosphatemia also promotes 1α-hydroxylase production and thereby increases $1,25(OH)_2D$.[16, 48]

$1,25(OH)_2D$ stimulates proximal intestinal calcium and phosphate absorption, and, together with PTH, increases distal renal tubular calcium reabsorption. Both calcium and phosphate absorption occur via saturable and nonsaturable pathways across the intestinal epithelium. $1,25(OH)_2D$ mediates transcriptional and possibly rapid nontranscriptional regulation of calcium absorption via mechanisms that remain to be clarified.[49] 1,25(OH)2D also regulates the sodium dependent type II phosphate cotransporter NaPi-IIb in the intestine.[50] Details of these mechanisms are beyond the scope of this review.[49, 50]

25(OH)D causes a net increase in bone density by promoting osteoblast differentiation and mineralisation following intracellular conversion to $1,25(OH)_2D$.[51] $1,25(OH)_2D$ and PTH stimulate osteoblast secretion of receptor activator of nuclear factor-kB ligand (RANKL), which in turn induces osteoclastogenesis and osteoclastic bone resorption and calcium mobilisation.[16, 52] This effect is attenuated by increasing levels of 25(OH)D.[51] $1,25(OH)_2D$ promotes the secretion of FGF23 from osteocytes, and this inhibits 1α-hydroxylase and upregulates 24-hydroxylase. PTH secretion is inhibited by both elevation of calcium and directly by $1,25(OH)_2D$, completing the negative feedback endocrine loop.[16, 53]

There is a wealth of human clinical data regarding the integral role of vitamin D in the maintenance of skeletal health. Osteomalacia, and rickets in children, are conditions characterised by a defect or delay in bone mineralisation, respectively. Typically, 25(OH)D levels in patients with osteomalacia are less than 20 nmol/L (8 ng/mL), and consequent calcium and phosphorus deficiency result in failure of organic osteoid mineralisation with bone pain or deformities.[51] Originally recognised in 1650 by Francis Glisson, its incidence increased dramatically during the industrial revolution in the 19th century.[5] Cod liver oil, one teaspoonful of which contains about 375 IU of vitamin D, was recognised as a folk remedy for rickets in infants.[11] Following the fortification of foods with vitamin D in the early 20th century, the incidence of rickets fell markedly.[5]

Osteoporosis is a condition of bone microarchitectural disruption with increased fragility but without an increase in unmineralised osteoid, and it is defined by a reduction in BMD at any major skeletal site of more than 2.5 standard deviations below the mean for young normal adults (a T score ≤ -2.5).[54] Though believed to be multifactorial in origin, vitamin D deficiency with secondary hyperparathyroidism, which increases bone resorption and results in cortical bone thinning, contributes to these pathological changes. Vitamin D supplementation at doses of 700–800 IU/day has been shown to reduce loss of BMD,[55] and in multiple randomised controlled trials (RCTs) to reduce the risk of vertebral and nonvertebral fractures[55–60]; however, most of these trials also entailed calcium supplementation in the same arm. A recent analysis of 12 RCTs of patients receiving vitamin D supplementation for nonvertebral fracture prevention found an optimal benefit at a serum 25(OH)D level of 75–110 nmol/L.[61] Trials of vitamin D alone have provided conflicting results,[60, 62–65] but the doses of vitamin D in the negative studies may have been too low to provide true benefit for fracture prevention. Observational studies corroborate a positive correlation between 25(OH)D levels and BMD, with maximal suppression of PTH,[66, 67] bone turnover markers[67] and pathological osteoid accumulation[68] with 25(OH)D levels above 75 nmol/L.

Vitamin D deficiency has also been associated with reduced muscle strength and appendicular muscle mass, muscle pain, increased body sway and risk of falls in observational studies.[52, 69, 70] In myocytes, vitamin D exerts VDR-mediated genomic regulation of expression of calcium pumps, calcium binding and cytoskeletal proteins, and phosphate metabolism.[71] Furthermore, rapid and direct nongenomic effects on calcium uptake via G-protein-mediated activation of phospholipase C, and

mitogen-activated protein kinase (MAPK) pathways, have been recognised.[44, 71] Vitamin D deficiency results in atrophy of fast twitch type II muscle fibre atrophy,[71] which are the first group of muscle fibres recruited during postural correction. Interventional trials assessing for changes in muscle strength and function using vitamin D have found conflicting results due to heterogeneity in dosage of vitamin D administered and levels attained,[72] but overall an improvement in hip muscle strength and reduction in falls has been demonstrated.[69, 72]

NONCLASSICAL ACTIONS OF VITAMIN D

A series of discoveries charting the anatomical distribution of vitamin D axis components have led to a paradigm shift from a purely endocrine action to intra-organ autocrine, paracrine and intracrine roles. First, the VDR has been isolated in tissues other than the intestinal epithelium, distal renal tubules and osteocytes, including the adrenals, parathyroids, heart, placenta, pituitary gland, ovary, testis, mammary glands, skin, hepatocytes, biliary epithelial cells, promyelocytes, thymus, lymphocytes and colon.[71, 73–77] Second, 1α-hydroxylase is also expressed in the colon, skin, lymph nodes, pancreas, adrenal medulla, brain and monocytes and macrophages, demonstrating the capacity for local production of 1,25 $(OH)_2D$.[78, 79] Third, though DBP is predominantly produced in the liver, mRNA for DBP gene transcripts have been noted in rat kidney, testis, abdominal fat, yolk sac, duodenum and ileum.[80] In fact, some authors have estimated that only about 5% of serum 25(OH)D is involved in the endocrine vitamin D system, with about 85% involved in autocrine and paracrine functions in local target tissues.[81]

Recent research has analysed effects of the vitamin D axis on cardiovascular disease, renal disease, glucose control, infection cancer, asthma and chronic obstructive pulmonary disease, cognitive and psychiatric disease, and autoimmune diseases including multiple sclerosis and IBD. To date, clinical data suggesting an inverse correlation between vitamin D levels and the incidence or risk of these conditions have been largely observational, and hence limited by multiple confounding factors.[5, 82] *In vitro* and animal *in vivo* data support a beneficial role for vitamin D supplementation or VDR agonism in cardiac hypertrophy, atherosclerosis,[83] hypertension, diabetic nephropathy,[84–86] colon,[87–89] breast[90, 91] and prostate cancer,[92–97] asthma and chronic obstructive pulmonary disease.[98–101] There are, however, very few prospective RCTs in humans demonstrating this benefit. The best evidence to date has been in pulmonary tuberculosis and diabetic nephropathy. An RCT demonstrated the superiority of high-dose vitamin D3 supplementation in combination with antituberculous therapy over no supplementation for treatment of pulmonary tuberculosis,[102] and a recent multi-national trial demonstrated that the addition of the VDR agonist, paricalcitol, to inhibition of angiotensin-converting enzyme further reduced albuminuria in patients with diabetic nephropathy.[103] Detailed reviews on the involvement of the vitamin D axis in various organ systems are presented elsewhere.[16, 82, 83, 87, 104–111]

VITAMIN D IN IBD

Vitamin D deficiency is more common in adults and children with IBD, especially CD, than healthy controls,[112–117] and correlates with a poorer health-related quality of life.[112] Many factors are likely to contribute to this, including malabsorption secondary to mucosal disease or surgical resection, and reduced sunlight exposure, physical activity and dietary intake. An active inflammatory state, which results in reduced hepatic production of DBP, may cause a reduction in total 25(OH)D levels,[33] but this has not specifically been studied in patients with IBD.

The relationship between the vitamin D axis and IBD appears to be a multi-faceted one, comprising maintenance of musculoskeletal health, and possibly control of disease activity through immunomodulation, and modification of the risk of IBD-associated malignancy.

Vitamin D and musculoskeletal health in IBD

The prevalence of low BMD is greater in patients with IBD than in healthy controls. It is estimated that 22–77% of patients with IBD have osteopenia, and 12–41% have osteoporosis.[118, 119] Most studies have reported that patients with CD have a greater prevalence of low BMD than those with UC, but some other studies have found similar rates.[120] Bone loss occurs in both cortical and trabecular regions, though the former predominates in CD.[119] Though glucocorticoid use is the most well-recognised risk factor for osteoporosis in IBD, reduced BMD is observed in patients with IBD in the absence of steroid use.[119] Other risk factors include older age, postmenopausal status, smoking, low body mass index, reduced physical activity, malnutrition and low vitamin D status.[118–120] A chronic inflammatory state, with effects on osteoblast and osteoclast function mediated by cytokines such as TNF-α, IL-6 and IL-1β, likely contributes to bone loss.[121, 122] The risk of low-trauma fractures in patients with CD is estimated to be increased by about 30%, and in UC about 20%, compared with controls.[123–125] The

American Gastroenterology Association, the American College of Gastroenterology and British Society of Gastroenterology recommend BMD testing in patients with IBD aged 60 and above, cumulative exposure to glucocorticoids for ≥ 3 months, low BMI, family or personal history of low-trauma fractures, or hypogonadism.[125–127] However, adherence to these recommendations by clinicians is suboptimal.[120]

There is an absence of controlled prospective trials for fracture prevention specifically in patients with IBD. However, it is recommended that, along with control of disease activity, encouragement of physical activity and cessation of smoking, vitamin D and calcium supplementation should be given to those at moderate or high risk of fracture, and antiresorptive therapy in those at high risk.[125, 126] In one small prospective trial, 1,25 $(OH)_2D_2$ supplementation reduced markers of bone turnover in patients with active CD.[128] In another prospective trial in the early 1990s, 25(OH)D administration in 75 patients with CD for 1 year reduced BMD loss as measured by distal forearm absorptiometry.[129] Bone protective therapies are underutilised in patients with IBD. One large review of over 2000 patients from 7 centres in USA found that only 59% and 75% of osteoporotic IBD patients received calcium/vitamin D supplementation and bisphosphonates, respectively.[120]

Children and adults with IBD also have reduced muscle mass.[130, 131] Sarcopenia is reported in 60% of patients with CD.[131] Increased apoptosis has been demonstrated in muscle biopsies in patients with IBD,[132] but the reasons for this have not yet been fully elucidated. Extrapolating from known effects of vitamin D insufficiency on muscle tissue, the optimisation of vitamin D status in patients with IBD may serve to preserve muscle health.

Vitamin D as an immunomodulator in IBD

There are accumulating epidemiological, physiological, genetic and clinical data for a role of vitamin D in immunomodulation in IBD (Table 1).

Epidemiological associations. There is a correlation between frequency of IBD and potential exposure to sunlight as indicated by distance from the equator.[133, 134] The incidence and prevalence of IBD is higher in northern Europe, North America, Australia and New Zealand than Asia.[1, 2, 135, 136] Also, the incidence of IBD in the Indian Subcontinent is low, but migrants to developed countries at Northern latitudes have a greatly increased risk of IBD.[137] In the Northern hemisphere, the onset of UC and exacerbations of CD are noted to peak in winter months.[138, 139] Nerich *et al.* have recently reported that a graded relative risk of CD incidence, but interestingly not UC incidence, correlated with areas of low sunlight exposure within France as ascertained from a population wide health insurance system database.[140] Furthermore, an analysis of 72 719 women aged 40–73 years enrolled in the Nurses' Health Study, using a predicted vitamin D level calculated from diet and lifestyle factors, has shown a reduced risk of CD (HR 0.54, 95% CI 0.30–0.99) and nonsignificantly reduced risk of UC (HR 0.65, 95% CI 0.34–1.25) in women with the highest quartile of vitamin D compared with the lowest quartile.[141]

The interpretation of the associations between vitamin D levels and sunlight exposure on the one hand and the incidence of IBD on the other is confounded by numerous factors, and a causal relationship cannot be assumed. Nonetheless, the data do provide a foundation for the investigation of the physiological connection between the vitamin D axis and inflammation in IBD.

Evidence for involvement in inflammation and immunomodulation. Both UC and CD are characterised by a dysregulated mucosal immune response to intestinal microorganisms in a genetically susceptible host. Fascinating insights ascertained from characterisation of VDR and other vitamin D axis components in the gastrointestinal mucosa, as well as genetic associations, provide evidence for the potential involvement of vitamin D at several stages of initiation and perpetuation of inflammation in IBD (Figure 2) as follows.

Maintenance of epithelial barrier: The columnar epithelial monolayer lining the gastrointestinal tract from the stomach to the rectum acts as a crucial interface between the mucosa and lumen, serving as a physical barrier as well as antigen presenter and immune regulator.[142, 143] Epithelial cells are connected by intercellular junctions, comprising tight junctions and adherens junctions, collectively referred to as the apical junctional complex, and desmosomes.[143] Altered intestinal permeability, resulting from defects in these junctions, may predispose to inflammation. Claudin-2, a pore-forming transmembrane protein that forms part of the tight junction, has been implicated in the pathogenesis of IBD.[143] Phosphorylation and expression of claudin-2 is stimulated by signal transducer and activator of transmission (STAT) 1 and STAT3, which is induced by IFN-γ, which is in turn inhibited by the protein tyrosine phosphatise N2 (PTPN2).[144] The gene coding for PTPN2 is a high-risk

Table 1 | Evidence for vitamin D as an immunomodulator in IBD

Epidemiological associations	Incidence and prevalence of IBD increase at latitudes away from equator Incidence and prevalence higher in northern Europe, North America, Australia and New Zealand than Asia Migrants from Indian subcontinent to northern latitudes are at increased risk of IBD Onset of UC and exacerbations of CD peak in winter months Lower risk of CD and probably UC with higher predicted vitamin D level
Physiological evidence (mainly *in vitro* and animal data)	
Maintenance of epithelial barrier	May inhibit claudin-2 Promotes tight junction proteins Zo-1, occluding and E-cadherin
Innate immune response	Promotes NOD2 gene transcription Stimulates cathelicidin production by macrophages Reduces PBMC proliferation and TNF-α secretion Inhibits dendritic cell maturation and production of IL-12 Stimulates IL-10 production
Adaptive immune response	Inhibits T-cell production of IFN-γ, IL-17 and IL-21 Stimulates CTLA-4 and FoxP3 In mouse colitis models, reduces IL-6, IL-17, IL-12p70, IL-23p19; and stimulates IL-10, TGF-β, FoxP3, CTLA-4, IL-4, GATA3
Genetic Associations	
VDR polymorphism	*Taq*I *tt* genotype over-represented in European and New Zealand cohorts
DBP polymorphism	Gc-2 allele underrepresented in patients with IBD
Clinical evidence	Alfacalcidol may be superior to cholecalciferol in reducing CDAI and CRP over 6 weeks.[128] Nonsignificant reduction in risk of relapse (13% vs. 29% at 12 months, $P = 0.06$) with 1200 IU cholecalciferol daily compared with placebo in 96 patients in clinical remission.[183] Significantly improved clinical symptom score in 15 patients with CD given 10 000 IU oral vitamin D daily compared with patients given 1000 IU vitamin D daily at week 26.[185]

DBP, vitamin D-binding protein; VDR, vitamin D receptor.

locus for IBD [145] and type 1 diabetes mellitus and is particularly associated with colonic CD and UC.[146] A recent ChIP-sequence genomic map has identified the gene bound by the VDR in CD as *PTNP2*.[42] Hence, 1,25 $(OH)_2$D-VDR complex-induced *PTPN2* expression may inhibit epithelial barrier pore formation and altered intestinal permeability.

In a mouse model of colitis, dextran sodium sulphate administration was demonstrated to reduce transepithelial electrical resistance and expression of the tight junction proteins Zo-1 and occludin prior to ulcers and clinical signs to a greater extent in VDR knockout (KO) mice than wild-type mice.[147] Furthermore, administration of 1,25$(OH)_2D_3$ enhanced Zo-1 and E-Cadherin expression in CaCo-2 cell cultures and enhanced epithelial reconstitution following injury.[147]

Innate immune response: Intestinal epithelial, dendritic cells and macrophages (collectively referred to as antigen presenting cells) express pattern recognition receptors (PRRs), which enable continuous monitoring of luminal contents for commensal and pathogenic organisms through the recognition of conserved structures called pathogen-associated molecular patterns (PAMPs).[148] Examples of PRRs include toll-like receptors (TLRs), nucleotide-binding domain and leucine-rich repeat-containing receptors (NLRs), C-type lectins and retinoic acid-inducible gene I-like receptors.[148] The protein NOD2 (CARD15), part of the family of NLRs, recognises modified muramyl dipeptide, a PAMP which is the lysosomal breakdown product of bacterial peptidoglycan.[148, 149] More recently, viral elements have also been shown to induce NOD2.[150] NOD2 has been associated with numerous regulatory roles in the mucosal immune system, with evidence for Paneth cell antimicrobial peptide generation, negative regulation of TLR signalling and hence induction of tolerance, and promotion of T-cell interleukin (IL)-10 expression.[145] The *NOD2* gene is the locus associated with the highest risk for CD so far identified [151] and *NOD2* mutations have been correlated with fibrostenosing CD. Significantly, dendritic cells, macrophages and intestinal epithelial cells express VDR,[152, 153] and 1,25$(OH)_2D_3$ has been shown to promote transcription of the *NOD2* gene,[154] highlighting an important link between the vitamin D axis and pathogenesis of IBD.

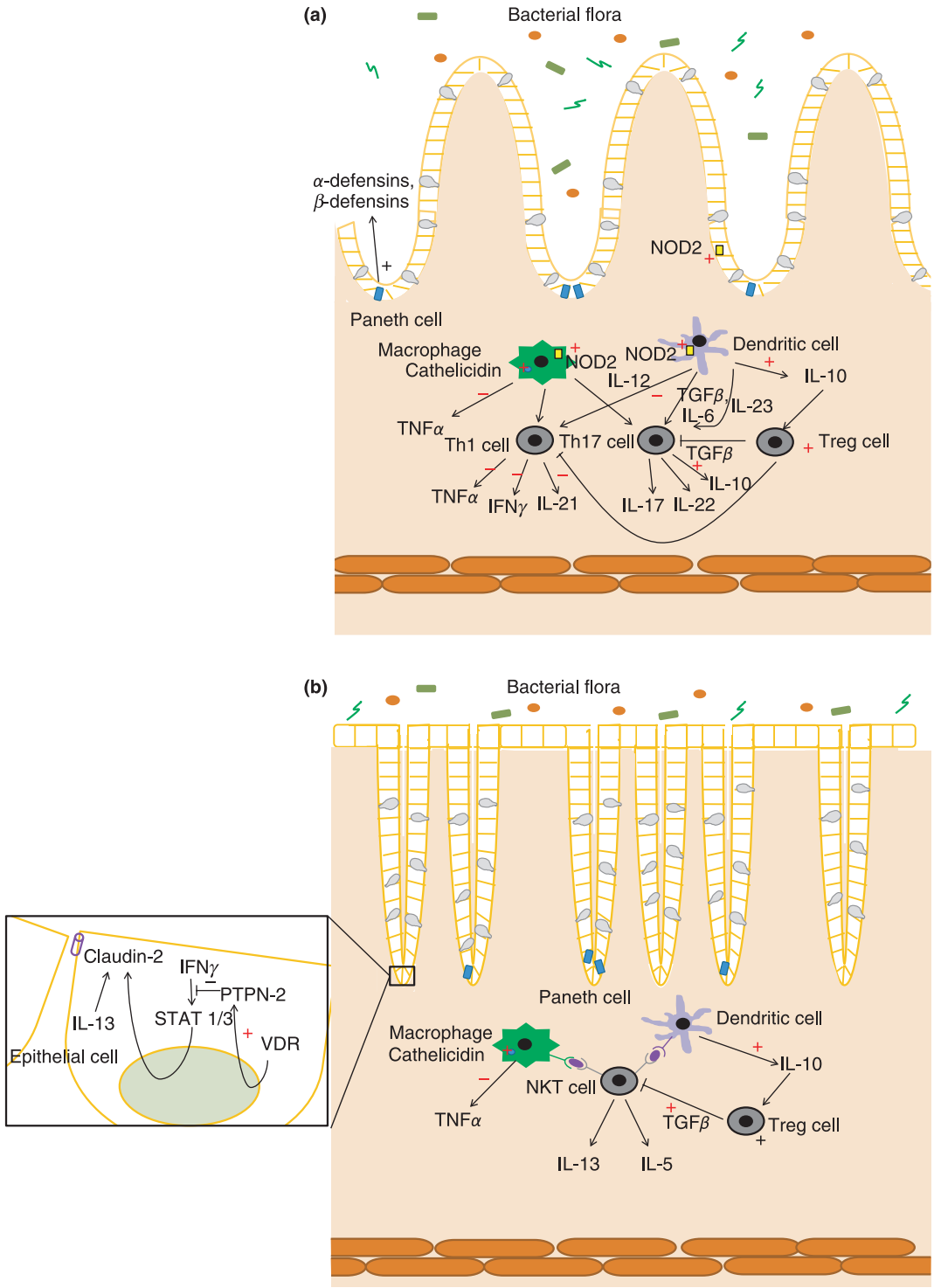

Figure 2 | Postulated immunomodulatory role of vitamin D in IBD. (a) Crohn's disease. Vitamin D has been shown to promote transcription of the intracellular pattern recognition receptor NOD2 and inhibit pro-inflammatory cytokine production by macrophages and Th1 cells (TNF-α, IFN-γ and IL-21). (b) Ulcerative colitis. In both Crohn's disease and ulcerative colitis, vitamin D promotes intracellular bacterial degradation via cathelicidin, promotes the regulatory cytokine IL-10 secretion and regulatory T-cell function and potentially inhibits colonic crypt epithelial barrier pore-forming claudin-2 via PTPN2. A red '+' indicates a positive effect of vitamin D, and a red '—' indicates an inhibitory effect of vitamin D.

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NOD2 has also been demonstrated to potentiate autophagy, the process by which damaged organelles, proteins and intracellular microorganisms are removed through engulfment into an autophagosome and lysosomally degraded.[155] Autophagy-related 16-like 1 (ATG16L1) is a protein that forms an integral component of this process, and its recruitment is mediated by NOD2. Deficiency of ATG16L1 results in an exaggerated inflammatory response,[155] and the gene encoding it, *ATG16L1,* has been identified as another major CD susceptibility locus from genome wide association studies (GWAS).[156] Hence, vitamin D signalling may indirectly promote regulation of inflammation through NOD2 and autophagy.[157]

Vitamin D also directly participates in autophagy by potently stimulating cathelicidin, an antimicrobial peptide produced by macrophages.[157] Cathelicidin plays an important role in defence against intracellular organisms, particularly mycobacteria.[153] The expression of cathelicidin is significantly increased in inflamed and non-inflamed mucosal biopsies from patients with UC, but unaltered in patients with CD, which may imply a defect in mucosal defence in the latter condition.[158] In human monocyte cultures, *E. coli* DNA induced expression of cathelicidin, a process dependent on TLR9 and MyD88 signalling.[159] Furthermore, cathelicidin expression is increased in mice administered intra-colonic bacterial DNA, and cathelicidin KO mice develop more severe DSS-induced colitis than wild-type mice.[159] In addition, intra-rectal cathelicidin administration ameliorated DSS-colitis.[160]

TNF-α is a major cytokine implicated in inflammation and cytotoxicity in both CD and UC and is increased in the lamina propria in both conditions.[145] TNF-α is believed to be primarily derived from macrophages in UC, but other cells within the lamina propria including T helper (Th) 1 cells contribute in CD.[145] In peripheral blood mononuclear cells derived from patients with IBD, proliferation and secretion of TNF-α are reported to be significantly reduced by administration of the VDR agonist KH 1060.[161]

The influence of vitamin D in the proliferation and maturation of APCs also extends to dendritic cells, the maturation of which is inhibited by both $1,25(OH)_2D_3$ [162] and its synthetic analogues.[163] Recent data from microarray studies show that $1,25(OH)_2D$ upregulates the expression of immune tolerogenic genes in dendritic cells.[164]

Adaptive T-cell response: Perhaps, the largest body of *in vitro* and animal *in vivo* evidence for an immunoregulatory role for vitamin D in IBD regards the adaptive T-cell response. APCs activate T-cell responses through direct interaction between MHC class I and II receptors and the T-cell receptor (TCR), in the presence of co-stimulatory signals (CD 80/86 and CD40 on APCs and CD28 and CD40L on T cells). CD is characterised by a Th1 and Th17 $CD4^+$ response, whereas UC comprises a Th2-like response.[165] The Th1 cell response and secretion of IFN-γ is induced by IL-12, which is derived from dendritic cells secondary to PRR signalling.[145] The Th17 response and production of IL-17 is stimulated by TGF-β and IL-6, in the presence of IL-23, which are also derived from APCs.[145, 165] In the absence of IL-23, Th17 cells produce IL-10, an anti-inflammatory cytokine.[165] In contrast, the UC mucosal immune response predominantly consists of natural killer T cell production of IL-13 and, to a lesser extent, IL-5.[165] Th1 and Th17 cells are also noted to be increased in number in the mucosa in UC.[166] Recently, IL-21, produced by Th1 and T follicular helper cells, has been recognised to promote Th17 cells and autoimmunity, as well as germinal centre B cells.[167] IL-21 is overexpressed in inflamed mucosa in CD and UC.[168]

VDR is expressed on activated T cells.[167] $1,25(OH)_2D_3$ has been shown to inhibit dendritic cell production of IL-12,[162] and $CD4^+$ T-cell production of IFN-γ, IL-17 and IL-21.[167] In addition, $1,25(OH)_2D_3$ stimulates expression of dendritic cell production of IL-10,[162] and T-cell levels of CTLA-4 (an inhibitory co-stimulatory signal) and FoxP3 (a lineage specification factor of regulatory T cells), further enhancing its anti-inflammatory effect.[167]

Pathogen-free VDR/IL-10 double KO mice develop fulminant DSS-induced colitis, compared with lack of colitis seen in pathogen-free IL-10 KO mice.[169] VDR KO mice also have reduced IL-10 and anti-inflammatory intra-epithelial $CD8\alpha\alpha$ lymphocyte levels.[169] IL-10 KO mice develop worse colitis and have a lower survival when fed a vitamin D deficient diet.[170] Vitamin D supplementation ameliorates and blocks the progression of colitis in IL-10 KO mice.[170] In 2,4,6-trinitrobenzenesulfonic acid-induced colitis mouse models, treatment with $1,25(OH)_2D_3$ reduces expression of IL-6, IL-17, IL-12p70 and IL-23p19 and increases expression of regulatory T-cell markers IL-10, TGF-β, FoxP3, CTLA4 and Th2 markers IL-4 and GATA3.[171] The experimental VDR agonist, BXL-62, inhibits DSS-induced colitis in mice.[172]

In PBMCs of humans with IBD, BXL-62 reduces pro-inflammatory cytokines TNF-α, IL-12/23p40, IL-6 and IFN-γ, both at mRNA and protein level.[172] In peripheral

blood $CD4^+$ T cells isolated from patients with IBD, 1,25 $(OH)_2D_3$ reduces IFN-γ and increases IL-10 production, alone and in combination with dexamethasone.[173]

Similar benefits have been reported in experimental models of other Th1-mediated autoimmune diseases, including multiple sclerosis, type 1 diabetes and rheumatoid arthritis.[174–176]

Genetic associations. Genetic polymorphisms in components of the vitamin D axis have been associated with IBD risk. Genome screening in Caucasians suggests the *Taq*I *tt* genotype is the VDR genotype over-represented in CD, with a prevalence of 22% and an odds ratio of 1.99 (95% CI 1.14–3.47; $P = 0.017$).[177] This increased frequency was replicated but limited to males with IBD in two other cohorts.[178, 179] Interestingly, immune modulation by vitamin D may be associated with the VDR polymorphisms. VD3 supplementation of antituberculosis therapy was only significantly superior in patients with the *Taq*I *tt* genotype.[102] A study in Iranian IBD patients, contrastingly, revealed an association with the *Fok*I *f* allele.[180] A further large study in Irish IBD patients found no statistically significant association with VDR genotype.[181]

Recently, a reduced frequency of Gc-2 alleles was reported in 636 IBD patients compared with 248 non-IBD controls, with a significant association for both CD and UC patients.[182] The mechanism by which this allele may reduce risk of IBD is uncertain. However, given that the Gc-2 allele confers a lower affinity for 25(OH)D and $1,25_2$(OH)D than Gc-1f and Gc-1s alleles, one may speculate that vitamin D metabolites are less freely available for immunoregulatory functions with the latter alleles in patients with IBD.

Clinical evidence for vitamin D as an immunomodulator in IBD. Small human clinical trials have suggested that vitamin D supplementation may have immunomodulatory activity in IBD. In a nonblinded trial of 37 patients with CD in clinical remission as defined by Crohn's Disease Activity Index (CDAI) <150, 18 patients administered 0.5 μg alfacalcidol ($1(OH)D_3$, a vitamin D analogue) had a superior improvement in CDAI and CRP over a 6-week period compared with 17 patients given 2000 IU cholecalciferol over a 6-week period.[128]

A randomised controlled trial of 94 CD patients with steroid-free remission (CDAI < 150, normal CRP and normal albumin) recently demonstrated a nonsignificant reduction in risk of relapse (13% vs. 29% at 12 months, $P = 0.06$) with 1200 IU cholecalciferol daily compared with placebo.[183] Somewhat surprisingly, a subgroup analysis of peripheral blood mononuclear cells isolated from CD patients treated for 26 weeks with cholecalciferol ($n = 10$) demonstrated increased IL-6 production and $CD4^+$ T-cell proliferation compared with placebo ($n = 10$).[184] This apparently paradoxical finding in the presence of clinical improvement may be explained by a dual function of the IL-6 cytokine family, which may also be cytoprotective via downstream effects under certain conditions as well as being pro-inflammatory.[145]

More recently, findings from a small trial of 15 patients demonstrated a significantly improved clinical symptom score in patients with CD given 10 000 IU oral vitamin D daily compared with patients given 1000 IU vitamin D daily at week 26.[185]

Interestingly, VDR mRNA expression is reduced in colonic biopsy specimens in patients with CD and UC,[186] and immunohistochemically localised VDR protein is less frequently noted in colonic specimens from patients with UC than normal controls.[187] Furthermore, vitamin D3 supplementation has been shown to induce VDR mRNA expression in a variety of tissues.[188, 189]

Vitamin D in chemoprevention

Patients with IBD are at increased risk of colorectal cancer, arising as a result of chronic inflammation.[190] Biological plausibility for a potential role for vitamin D in chemoprevention for colorectal cancer arises from data *in vitro* demonstrating expression of VDR in colon and rectal cells, particularly cancer cell lines.[77, 87, 89, 191] Vitamin D has been shown to inhibit cancer cell growth and proliferation through regulation of growth factors and increasing apoptosis.[192, 193] VDR KO and vitamin D deficient mice are at increased risk for colonic epithelial hyperplasia and cancer.[194–196]

Epidemiological studies mostly have shown an inverse correlation between risk of colorectal cancer and serum 25(OH)D levels, with the highest quintiles offering protection from colorectal cancer of up to 50% compared with the lowest quintile.[197–199] However, the quality of some of these studies has been questioned, and some smaller studies have shown no such benefit.[197, 200]

In the largest reported prospective trial to date, the Women's Health Initiative study of 36 282 postmenopausal women did not show a reduced risk of colorectal cancer with administration of 400 IU cholecalciferol combined with 1000 mg calcium carbonate daily for 7 years.[201] However, this null result may be explained by a relatively low dose of vitamin D along with a relatively short duration compared with the long period over

which colorectal cancer develops. No randomised controlled trials investigating vitamin D alone have been yet conducted.

Furthermore, it is unclear whether any potential protective effect for non-IBD-associated colorectal cancer may extend to IBD-associated malignancy. In the absence of robust data, it is premature to recommend vitamin D supplementation specifically for chemoprevention.

CLINICAL CONSIDERATIONS FOR VITAMIN D THERAPY

The optimal management of vitamin D status, along with its reported clinical effects, is perhaps one of the more vigorously debated and controversial areas in medicine today. To develop recommendations for therapeutic targets and means of optimisation, determinants of vitamin D status, evidence for current therapies and effectiveness of intervention, and toxicity need to be considered (Table 3), while allowing for evolutionary and physiological principles for the pleiotropic involvement of the vitamin D axis.

Determinants of vitamin D status: genetic and environmental

As outlined previously, 25(OH)D is considered the best measure of vitamin D status as it is the main storage and circulating form of vitamin D. 25(OH)D levels are determined by numerous variables (Table 2), both genetic and environmental, which have the potential to confound epidemiological observational studies. The total variation explained by genetic factors is less than that due to environmental factors.[202] Moreover, despite consideration of all of these factors, much of the variation in 25(OH)D levels in the general population remains unexplained.[5]

Table 2 | Determinants of 25(OH)D level[5, 197, 221]

Environmental/Modifiable	Genetic/Nonmodifiable
Sunlight exposure	Skin type
Dietary/supplemental vitamin D intake	Race
Dietary/supplemental calcium intake	Age
Obesity	*GC* (Vitamin D-binding protein)
Physical activity	*CYP24A1* (24 hydroxylase)
Malabsorption	*DHCR7* (7-dehydrocholesterol reductase)
Liver disease	*CYP2R1* (25 hydroxylase)
Kidney disease	
Parathyroid hormone concentration	
Anticonvulsant therapy	

Vitamin D deficiency, insufficiency, sufficiency, toxicity and supplementation

Current definitions of vitamin D deficiency, insufficiency and optimal levels are based largely on observations of PTH levels and bone health, but universal consensus remains elusive. The commonly quoted definition of vitamin D deficiency is a 25(OH)D level less than 25 nmol/L (10 ng/mL), the level that places an individual at highest risk for development of osteomalacia.[203] However, the Endocrine Society has recently published a less conservative level of less than 50 nmol/L (20 ng/mL) as vitamin D deficiency.[4] Vitamin D sufficiency is commonly quoted as 75 nmol/L or more, based on suppression of PTH and lack of pathological osteoid accumulation in bone.[66, 68] However, many experts classify vitamin D sufficiency as 50 nmol/L or more.[203] The intermediate value, which variably becomes 25–50 nmol/L (or 25–75 nmol/L) in most cases, is referred to as vitamin D insufficiency.

Excess 25(OH)D levels may result in vitamin D toxicity, or hypervitaminosis D. The most easily recognisable manifestation of vitamin D toxicity is hypercalcaemia. However, there have been very few reports in the literature of hypercalcaemia from vitamin D ingestion only, with most occurring secondary to accidental overdose of very large quantities of vitamin D. The 25(OH)D level accounting for hypercalcaemia reported in these cases has varied from 320 to 1692 nmol/L, far in excess of levels noted in the population.[197] Such levels require the intake of oral supplementation of greater than 10 000–40 000 IU per day of vitamin D over prolonged periods.[197] There have been no reports of toxicity from UVB exposure alone.[197]

However, whether long-term exposure of lower doses of vitamin D may increase the risk of hypercalciuria and renal stones, nephrocalcinosis and potentially vascular calcification is uncertain and not adequately assessed in most short-term human trials to date.[197] Supporting these potential adverse effects is modelling from several observational and interventional trials demonstrating a 'reverse J' relationship between serum 25(OH)D levels and all-cause mortality.[197] An increase in mortality is seen with 25(OH)D levels below 50 nmol/L, a reduction between 50 and 75 nmol/L, and a slight upstroke in mortality at levels above 75–80 nmol/L.[197, 204, 205] For this reason, the Institute of Medicine of the National Academies recommend a relatively conservative dietary

Table 3 | Clinical considerations for vitamin D therapy

Units used for serum 25(OH)D concentration	ng/mL and nmol/L 1 ng/mL = 2.5 nmol/L
Units used for oral supplementation of vitamin D (cholecalciferol, ergocalciferol)	μg and IU 25 μg = 1000 IU
Serum 25(OH)vitamin D level: Controversial definitions (nmol/L)	
Deficiency	<25 (IOM) <50 (Endocrine Society)
Insufficiency	25 to <50 (IOM) 50 to <75 (Endocrine Society)
Sufficiency	≥50 (IOM) ≥75 (Endocrine Society)
Safety of vitamin D supplementation: levels of 25(OH)D at which toxicity is observed (nmol/L)	
Hypercalcaemia	>320 (case reports only)
Hypercalciuria and vascular calcification	Possibly >80–125 (poorly defined, wide inter-individual variation). Most likely with concurrent calcium supplementation.
Increase in all-cause mortality	Possibly >75–80 (conflicting studies)
Vitamin D intake recommendations (IU/day)	
Adults and children >8 years of age (general population)	
Daily allowance	600 or 800 if aged >70 (IOM) 1500–2000 (Endocrine Society)
Tolerable upper limit	4000 (IOM) 10 000 (Endocrine Society)
States of increased requirements	Pregnancy
	Obesity
	Underlying deficiency
Response to supplementation	
Healthy population	Highly variable: 0.57–1.9 nmol/L/μg vitamin D administered daily for 4–6 months
Crohn's disease, other malabsorptive states	About 30% lower, less if diseased jejunum
Obesity	About 50% lower
Mode of supplementation	Daily dosing may be superior to intermittent high dosing; latter preferred if adherence uncertain
Vitamin D formulations	Cholecalciferol (vitamin D3)
	Ergocalciferol (vitamin D2)
	Calcitriol (1,25(OH)vitamin D3); preferred in chronic kidney disease as does not require 1α-hydroxylation
Vitamin D receptor agonists	Paricalcitol
	Maxacalcitol
	Doxercalciferol
	Alfacalcidol
	Calcipotriol (topical)

IOM, Institute of Medicine; IU, international units.

allowance of vitamin D for the general population of 600 IU/day and tolerable upper limit of 4000 IU/day in all adults and children aged above 8 years of age.[197] This upper limit, however, does not apply to patients already deficient in vitamin D or those with increased requirements due to malabsorption. In contrast, the Endocrine Society recommends a daily intake of 1500–2000 IU/day for adults with an upper limit of 10 000 IU/day.[4]

It is unknown what level of 25(OH)D is required for optimisation of potential immunomodulatory effects of vitamin D *in vivo* in humans. Some authorities believe that a 25(OH)D level of over 75 nmol/L is required, extrapolating from anti-TB studies,[206] but no solid evidence in autoimmune disease is present.

Just as variation in levels of 25(OH)D exist in the general population, so does the response to supplementation of vitamin D. Trials for fracture prevention noted a variable increase in mean 25(OH)D levels with 400 IU cholecalciferol administration; an increment from 47 to 64 nmol/L over 12 months in one trial of 1144 nursing home participants,[62] but only 37–47 nmol/L over 24 months in another trial of 7073 community dwelling participants.[207] Five-month administration of 0, 1000 IU, 5000 IU and 10 000 IU cholecalciferol in 67 healthy

males with mean baseline 25(OH)D of about 70 nmol/L [208] demonstrated a dose response relationship, with significant increases obtained only by the higher two doses. Furthermore, the trajectory of rise in 25(OH)D plateaued in all groups, and no toxicity was noted in any patients. Mathematical calculation by this and other groups have noted an approximate increase in 25(OH)D level of 0.57–1.9 nmol/L/μg cholecalciferol administered daily.[208] Obesity, in increasing the pool of storage of 25(OH)D, limits the response to oral vitamin D, such that about twice the dose of vitamin D is required to obtain the same increase in serum 25(OH)D.[4] Furthermore, anticonvulsants increase catabolism of 25(OH)D, so patients on these therapies require additional dosing.[4]

Patients with CD may have an attenuated response to vitamin D therapy.[209] In a study of 37 patients with CD and 10 healthy controls, the increment in serum 25(OH) D_2 (not total 25(OH)D) was 30% less in the CD patients 12 h after an oral dose of 50 000 IU ergocalciferol.

It is as yet unclear if daily dosing of vitamin D is equivalent to intermittent administration of high-dose vitamin D, with the latter formulation designed for rapid repletion. A comparison of 400 IU twice daily (800 IU/day) or 97 333 IU of cholecalciferol every 4 months (total dose 292 000 IU in both groups) in 40 elderly women found a higher proportion of the daily dosing group (47%) achieved a 25(OH)D level of 75 nmol/L compared with 28% in the intermittent high-dose group after 12 months.[210] In a separate trial, no difference in target levels were found between the administration of 50 000 IU daily for 10 days (total dose 500 000 IU) or 3000 IU daily for 30 days followed by 1000 IU daily for 60 days (total dose 150 000 IU) at 3 months in 26 vitamin D deficient patients.[211] Single large doses of oral and intramuscular cholecalciferol (300 000 to 600 000 IU) have been described as being safe and effective, but transient hypercalciuria needs to be monitored.[212, 213] Intermittent large dosing may improve adherence outside the trial setting, so may be a reasonable alternative to daily dosing where this is a concern.

Some authors believe that cholecalciferol (VD3) supplementation, the naturally occurring form in humans, is more effective than ergocalciferol (VD2) for attainment of target 25(OH)D levels and skeletal protection.[11] Studies assessing this have been conflicting to date.[214–216] However, cholecalciferol has in recent years become more widely produced and available as commercial supplements worldwide, and supply of ergocalciferol has declined.

There is little evidence upon which a therapeutic plan can be based. However, a suggested approach to vitamin D supplementation in patients with IBD is illustrated in Figure 3.

Activated vitamin D3 (1,25 $(OH)_2$ D3) is not recommended for supplementation outside the chronic kidney

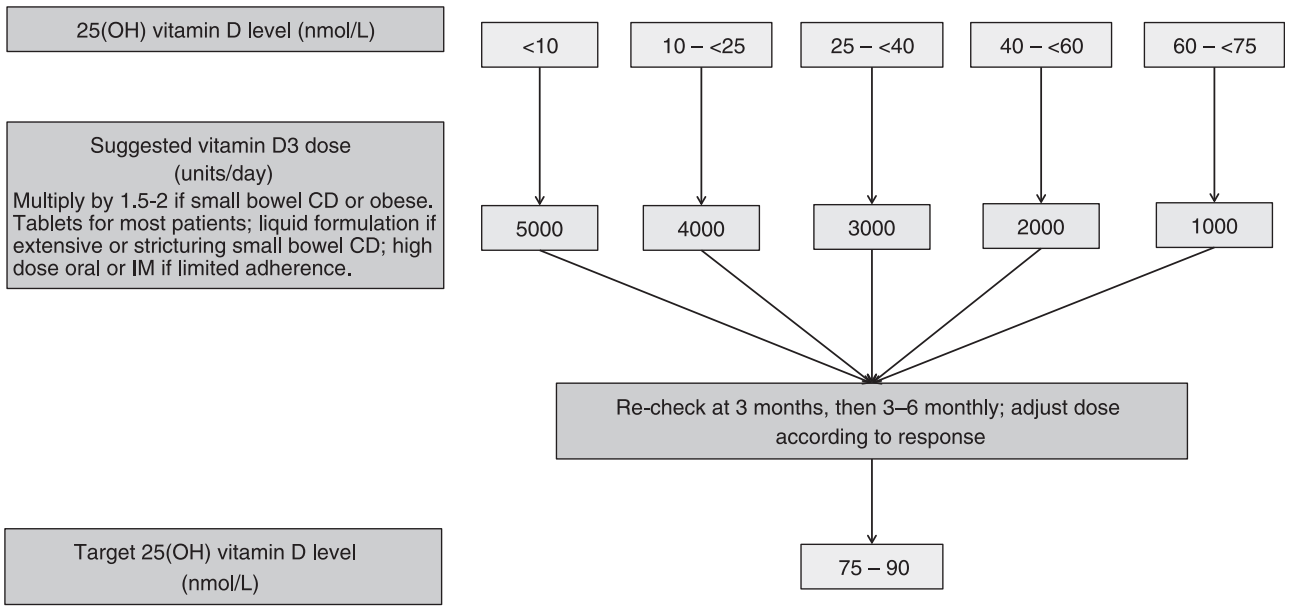

Figure 3 | Suggested algorithm for vitamin D supplementation in IBD. There is a wide inter-individual variation in response to vitamin D supplementation and adjustment according to level achieved is often required. The target 25 (OH) D level is based on current limited data and may evolve as more clinical evidence is obtained.

disease setting, as this metabolite is not available for intracrine or paracrine actions, and may enhance vascular calcification and hypercalcaemia.

In an attempt to increase specificity for vitamin D therapy for the parathyroid gland and reduce PTH without inducing hypercalcaemia, and hence to reduce morbidity associated with vascular calcification in chronic kidney disease, vitamin D receptor agonists have been developed. To date, the oral compounds apart from calcitriol to have been licenced and studied include paricalcitol, maxacalcitol (22-oxacalcitriol), which directly activate VDR, and doxercalciferol, and alfacalcidol (1α (OH)D), which require 25-hyroxylation to become active.[81] Whether these agents are able to be applied outside chronic kidney disease, with sufficient tissue activity in immune cells for instance, is unknown. Given that many of these cells express 1α-hydroxylase, the supply of 25(OH)D may be just as important or potent as direct activation by VDR agonists. Interestingly, a topical agent, calcipotriol, alone and in combination with betamethasone, is under clinical use for psoriasis, another Th1-mediated disease.[217] A more detailed discussion of VDR agonists is beyond the scope of this review and is presented elsewhere.[81, 218]

Vitamin D assays

A complicating factor in the study of vitamin D and its effects has been variability in accuracy and precision of assays for 25(OH)D. Currently, liquid chromatography-mass spectrometry (LC-MS) and high performance liquid chromatography (HPLC) assays are considered the most accurate and are increasingly available in laboratories worldwide.[197, 219] However, most literature in the past 20 years has quoted chemiluminescent assays or radioimmunoassays (still the most readily available), which are subject to more errors in observation and interpretation more than LC-MS and HPLC.[197, 220] To optimise assays, a Vitamin D External Quality Assurance Scheme (DEQAS), which monitors the performance of 25(OH)D assays of more than 700 laboratories worldwide on a quarterly basis, has been established.[197] This scheme uses the 'all laboratory trimmed mean' as the gold standard for assessing these assays.

CONCLUSION

Current understanding points to beneficial effects of vitamin D supplementation in patients with IBD in terms of bone and muscle preservation, reduction in inflammation and potentially reduced risk of cancer in patients with IBD. However, these beneficial effects must be balanced with potential adverse effects in the clinical setting. The precise thresholds of serum 25 (OH) vitamin D for beneficial and potentially adverse effects remain poorly defined in the literature. Until further evidence is available, we recommend to aim for a serum 25(OH) vitamin D level of 75 nmol/L. In a condition with a propensity to affect young people and a disproportionate effect on quality of life and productivity, the potential advent of inexpensive supplementary therapies presents an attractive option for ongoing research.

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