Vitamin D treatment for connective tissue diseases: hope beyond the hype?
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Abstract
The prevalence of vitamin D deficiency is increased among patients with CTDs. The active form of vitamin D (calcitriol) is a potent regulator of the immune system and may suppress inflammatory responses. This has led to claims that vitamin D may be a safe treatment, or a treatment adjunct, to reduce systemic inflammation in this patient population. It is important to note, however, that there is insufficient evidence from robust clinical trials to support these novel uses for vitamin D. In this review we examine the potential role of vitamin D as a treatment adjunct for CTDs. We will discuss how vitamin D may modulate the immune response and review the current evidence for using vitamin D to treat CTDs and their associated co-morbidities. We conclude that while there is much excitement about vitamin D in this context, further well-designed trials are needed to demonstrate its efficacy in the treatment of patients with CTDs.

Key words: vitamin D, systemic lupus erythematosus, connective tissue disease, inflammation, cardiovascular disease

Introduction
Vitamin D is a steroid hormone important for calcium homeostasis and the maintenance of bone health [1]. In humans, ∼80% of vitamin D is obtained by the photoconversion of 7-dehydrocholesterol into pre-vitamin D₃ by UV light on the skin [2, 3]. 7-dehydrocholesterol subsequently undergoes non-enzymatic transformation into vitamin D₃ (cholecalciferol). A second form, vitamin D₂ (ergocalciferol), is obtained principally from the diet [4]. Both ergocalciferol and cholecalciferol are relatively biologically inert. Activation occurs in a two-stage process: initially, 25-α hydroxylation within hepatic microsomes, resulting in the formation of 25(OH)D [5]. While 25(OH)D has some activity at the vitamin D receptor (VDR), the 1-α hydroxylated form [1,25(OH)₂D or calcitriol] has approximately 10 times greater potency. Although 1-α hydroxylase (CYP27B1) was first identified within the mitochondria of the kidney, other tissues contain this crucial enzyme [6].

Recently there has been interest in other roles of vitamin D, particularly in relation to inflammatory diseases [4]. The VDR has been identified in a number of tissues, including the skin and vasculature [7]. In the general population, large prospective observational studies have identified associations between vitamin D deficiency and a number of chronic illnesses [8].

The definitions of vitamin D deficiency and sufficiency remain controversial even within the general population. While a threshold of 20 ng/ml (50 nmol/l) has been proposed, based on the observation that serum PTH begins to rise at levels <20 ng/ml [9], demineralized osteoid is identified at post mortem with concentrations <30 ng/ml (75 nmol/l) [10]. A consensus opinion in 2005 suggests a target of 28–32 ng/ml (70–80 nmol/l) [11]. A number of high-risk groups have been reported, including pregnant women, older adults and various ethnic groups [12]. It is not known whether these different groups require different target vitamin D concentrations.
Low vitamin D has been reported in patients with CTDs, including SLE [13], primary SS [14] and idiopathic inflammatory myopathy (IIM) [15]. While the prevalence of vitamin D deficiency is well-recognized, its role in the development, progression and clinical manifestations of CTDs is not clear. Many rheumatologists remain uncertain about which patients should be tested, how deficiency should be treated and what benefits to expect from such interventions.

This review will assess the evidence for the use of vitamin D to treat CTD manifestations beyond its effects on bone health. We will focus primarily on data from observational and interventional studies in SLE, although other CTDs will also be considered.

Prevalence of vitamin D deficiency in SLE

Compared with healthy controls, patients with SLE have an increased risk of vitamin D deficiency. On reviewing studies that included a healthy control comparator group, lower vitamin D levels were reported in SLE patients in 12/14 (86%) such studies (see supplementary Table S1, available at Rheumatology Online). Conversely, Mandal et al. [16] found no difference in 25(OH)D levels between SLE patients and healthy controls, although there was marked vitamin D deficiency among the control group. Furthermore, 39% of the SLE patients were taking steroids and calcium/vitamin D supplements, which may have masked any differences [16]. Similarly, Stockton et al. [17] also found no difference in 25(OH)D between SLE patients and controls, which may be explained by 13/24 (54%) patients taking vitamin D supplements compared with 2/21 (10%) controls. Both of these studies may therefore have underestimated the prevalence of vitamin D deficiency in the patient populations.

Causes of vitamin D deficiency in SLE

The true relationship between vitamin D and inflammation remains to be determined. CTDs are chronic, often debilitating diseases with high levels of morbidity. In healthy subjects, reduced physical activity and reduced sun exposure (but not dietary intake) are important determinants of vitamin D status [18]. Vitamin D deficiency in the context of chronic illness may simply reflect reduced outdoor activity, and thus UV exposure, in these patients (reverse causation). This is relevant in SLE due to the photosensitive nature of the disease and the recommendations of chronic illness may simply reflect reduced outdoor activity (reverse causation). This is relevant in SLE due to the photosensitive nature of the disease and the recommendations of sunblock avoidance and high sun protection factor (SPF) sunblock use [19].

A further explanation for the association between vitamin D deficiency and autoimmune disease is that 25(OH)D may act as a negative acute phase reactant. In a meta-analysis, low serum 25(OH)D was reported following an acute event (including orthopaedic surgery and acute pancreatitis) in 6/8 studies, often in association with a decrease in serum albumin and an increase in CRP [20]. It is proposed that this decrease occurs due to reduced levels of the vitamin D binding protein [21].

Finally, it is plausible that vitamin D deficiency may drive the development of autoimmune disease and potentiate the inflammatory response. An early study by Kamen et al. (2006) identified lower serum 25(OH)D observed in recently diagnosed SLE patients compared with healthy controls and suggested that vitamin D deficiency may be a risk factor for the development of SLE. In this study, however, the patients already had SLE [22], a condition that often has prolonged delays in diagnosis. Furthermore, the difference in vitamin D levels was only statistically significant for Caucasian patients (62% of the study cohort). In terms of leading to the development of autoimmunity, a small cross-sectional study of European Americans found significantly increased vitamin D deficiency in ANA-positive vs ANA-negative healthy controls [23]. In a linkage analysis study, admission to hospital for vitamin D deficiency (including osteomalacia or rickets) was associated with increased future risk of developing a number of immune-mediated diseases, including RA, SLE and SS [24]. Other large prospective studies of women have not identified any association between vitamin D intake (as assessed by dietary questionnaire) and the risk of developing SLE [25, 26]. However, these observations may reflect the inadequacy of using dietary questionnaires to estimate vitamin D status, particularly given the importance of cutaneous synthesis of vitamin D.

There is evidence that vitamin D deficiency may have a role once autoimmunity has developed. In a study by Zold et al. [27], patients who progressed from a UCTD to a clearly defined CTD had significantly lower vitamin D levels than those that did not progress.

How might vitamin D deficiency lead to the development of SLE?

Polymorphisms in the vitamin D pathway

The VDR genotype may provide a link between low serum 25(OH)D levels and SLE. The Fokl polymorphism (rs2228570) is notable since the FF genotype was associated with low 25(OH)D levels in a small genetic study [28] and an increased risk of SLE in a separate larger study [29]. A further VDR polymorphism, Bsml (rs1544410), has also been associated with an increased risk of SLE in Asian subjects, although it is not clear whether this is independent of serum 25(OH)D levels [29]. If vitamin D status is partly dependent on genetic variation, then a fixed definition of deficiency across populations may not be appropriate. The normal range for 25(OH)D may need to be redefined on the basis of the VDR genotype.

Vitamin D and immunomodulation

Vitamin D has broad immunomodulatory effects across both the innate and adaptive immune systems (Fig. 1). Of particular relevance to SLE, calcitriol reduces anti-dsDNA antibody production by inhibiting B cell proliferation, by reducing differentiation into plasma cells and by B cell apoptosis [30, 31]. A decrease in anti-dsDNA has also been demonstrated in vivo after 4 weeks of...
high-dose cholecalciferol [32]. This effect was not due to changes in memory B cells alone. Increases in naive and regulatory T cells and decreases in Th1 and Th17 cells were also observed. Furthermore, in terms of T cells, in vitro studies have also shown that the VDR is upregulated following T cell activation and that calcitriol can polarize cells towards the Th2 phenotype [33]. Similarly, in a clinical study, high-dose cholecalciferol resulted in a reduction in the IFN-γ:IL-4 ratio, representing a Th1 to Th2 shift [34]. In healthy subjects, high-dose vitamin D also increases the number of FoxP3+ regulatory T cells [35].

In the innate immune system, dendritic cells (DCs) and monocytes express both the VDR and CYP27B1. The expression of CYP27B1 in monocytes, and thus local calcitriol concentration, is under regulatory control by other immune cells. The effect of calcitriol on innate immune cells is predominantly immunosuppressive, with reduced monocyte differentiation and DC maturation, downregulation of MHC II and CD80/86, leading to reduced T cell activation [36–39]. This has relevance for SLE, as calcitriol attenuates monocyte expression of MHC II, CD40 and CD86 in response to lupus serum [40]. In a study of patients with type 1 diabetes mellitus, vitamin D supplementation resulted in reduced differentiation of monocytes into DCs and the expansion of an intermediate cell type phenotypically similar to tolerogenic DCs [41]. In addition, toll-like receptor 9 (TLR9) senses hypomethylated DNA present in immune complexes and other sources, promoting an inflammatory response [42]. Vitamin D downregulates TLR9 expression in healthy human monocytes, resulting in reduced IL-6 production in response to TLR9 stimulation [43].

Much less is known about the effects of vitamin D on neutrophil function. In animal models, calcitriol reduces neutrophil recruitment, possibly via an effect on IL-8 [44]. Vitamin D may also have direct effects on neutrophil function, as neutrophils express VDR mRNA and show differential gene expression in response to 1,25(OH)2D3 [45]. The relevance of these changes remains to be examined in the clinical setting.

**Low vitamin D and lupus disease activity**

The relationship between 25(OH)D and lupus disease activity remains unclear. While some studies have demonstrated an association [16, 28, 46–50], others have not [23, 51–54]. This discrepancy cannot be clearly explained, but there are many potential confounding factors. As an example, the largest of these studies was conducted by Amital et al. [50] and comprised 378 patients from several European and Israeli cohorts. A modest association was seen between the vitamin D level at a single time point and disease activity ($r = -0.12$, $P = 0.018$). In this study, disease activity was not defined using a standardized scoring
Interventional studies of vitamin D in SLE

Only a small number of interventional studies have investigated the effect of vitamin D on disease activity in SLE, the majority of which have been inconclusive. The largest observational study of 763 SLE patients explored the relationship between changes in vitamin D and disease activity. While it was not a true interventional trial, deficient patients were treated with 50,000 IU ergocalciferol (plus 200 IU cholecalciferol) as per local guidelines. Although this could be considered a ‘high-dose’ regimen, the mean increase in serum 25(OH)D was not reported. The authors identified small changes in disease activity [reduction in Safety of Estrogen in Lupus Erythematosus National Assessment—SLEDAI] score of 0.22 (95% CI –0.41, –0.02) for a 20 ng/ml increase in vitamin D] and urinary protein:creatinine ratio [4% (95% CI 2, 5) decrease in PCR for a 20 ng/ml increase in vitamin D] in response to vitamin D therapy. The association between the change in serum 25(OH)D and disease activity was only apparent using a two-slope model and only when 25(OH)D was <40 ng/ml [57]. Such associations are also very modest and of uncertain clinical significance.

Two double-blind RCTs have also focused on the effect of vitamin D on laboratory markers of disease activity. Abou-Raya et al. [13] reported that 2000 IU/day for 12 months significantly reduced anti-dsDNA and anti-Sm titres and increased serum C4 compared with placebo. In this study, patients were allowed to continue baseline medication, but no details of any changes in medication over the 12 month trial were described. Furthermore, only a subset of the whole trial population was reported in the paper (in the intervention group, the change in SLEDAI is presented for 122/178 subjects). In contrast, a smaller study by Aranow et al. failed to find any change in expression of the IFN signature in response to vitamin D [58]. Although similar dosing regimens (2000 and 4000 IU/day) were used, the study was shorter (12 weeks), and again achieved incomplete vitamin D repletion (only 16/33 of treated patients at the end of the study). Recently another small high-dose crossover trial of 25,000 IU/month vs 300,000 IU loading and 50,000 IU/month also failed to show a change in disease activity. A limitation of this study was that only the high-dose regimen increased serum 25(OH)D levels, which may have been due to the high mean baseline 25(OH)D of 31.7 ng/ml [59].

A small trial randomized 40 adolescent SLE patients to 50,000 IU/week cholecalciferol or placebo and identified a significant difference in SLEDAI score and dsDNA positivity after 24 weeks [60]. The study does not clearly demonstrate that vitamin D therapy reduces disease activity; in the treated group, the SLEDAI score remained stable, but worsened in the placebo group.

These interventional studies highlight some of the difficulties in conducting clinical trials of vitamin D, including selecting the correct patient population, dose and treatment duration and determining the influence of potential threshold effects. Patients who are vitamin D deficient at baseline who receive adequate replacement may have the greatest benefit, pointing towards the need for a personalized approach. However, this hypothesis needs confirmation in well-conducted clinical trials.

SS

A cross-sectional study of 107 Turkish patients with SS found lower vitamin D levels in female but not male patients compared with controls [14]. This may reflect a true gender difference, although there were only 10 (9.3%) men in the study. In contrast, Agmon-Levin et al. [61] found no difference in serum 25(OH)D between SS patients and controls, although levels were lower in patients with peripheral neuropathy (18.6 vs 22.6 ng/ml) or lymphoma (13.2 vs 22.0 ng/ml). Furthermore, there is no evidence that genetic polymorphisms in the vitamin D pathway associate with the prevalence or severity of SS [62]. No studies have investigated the effect of vitamin D treatment in SS.

IIM

Little is known about the role of vitamin D in IIM. A single study of 149 IIM patients and 290 healthy subjects found a prevalence of vitamin D deficiency of 53–68% across the IIM subtypes compared with 21% in controls [15]. An inverse association between 25(OH)D and disease activity (assessed by physician global assessment) has been reported in JDM. However, the relationship was weak, with a 1 cm change in physician global assessment associated with only a 1.7 ng/ml change in 25(OH)D [63]. Similar to SS, there are also no associations between VDR polymorphisms and IIM. In a Hungarian study of 89 DM and PM patients, there were no differences in either VDR polymorphisms or haplotypes between the IIM patients and healthy subjects [64]. While there are also no intervention studies of vitamin D in IIM, a single in vitro study suggests that vitamin D may be able to modulate the inflammatory response. The cytokine CXCL10 is released from skeletal muscle in response to pro-inflammatory cytokines and is increased in the serum of IIM patients. VDR agonists can decrease CXCL10 secretion by human skeletal myocytes in response to stimuli [65].
SSc

The potential relationship between vitamin D and skin fibrosis is complex and beyond the scope of this review. Notably, the pro-fibrotic cytokine TGF-β1 is increased in the serum of vitamin D-deficient subjects, and the pro-fibrotic effect of TGF-β1 on epithelial fibroblasts is attenuated by exposure to 1,25(OH)2D3. Within fibroblasts, TGF-β1 signals via the phosphorylation of the Smad3 pathway. In animal models, vitamin D analogues reduce skin fibrosis via activation of the Th2 pathway.

A number of studies have identified that vitamin D deficiency is common in SSc. Amson et al. measured 25(OH)D in 327 European SSc patients and 141 healthy controls. In this study, vitamin D levels were significantly lower in patients compared with controls (13.5 ng/ml (s.d. 9.0) vs. 21.6 (9.7)) and were inversely associated with the severity of skin fibrosis. While reduced cutaneous synthesis due to skin thickening and reduced intestinal absorption have been postulated to contribute to vitamin D deficiency, analysis of vitamin D metabolites suggests that these processes actually remain intact. Anti-vitamin D antibodies are prevalent in SSc, although there is no evidence that they contribute to disease development or progression.

A small study of oral calcitriol in scleroderma spectrum disorders failed to show any benefit over placebo, although only 2/27 patients had SSc while 20 had morphea. Cutaneous calcinosis is an important feature of lcSSc. Given that vitamin D regulates serum calcium and mobilizes calcium from bone, it is important to better understand any potential role of vitamin D in SSc to ensure that vitamin D therapy does not exacerbate calcinosis.

Vitamin D and CTD-related co-morbidity

Cardiovascular disease

A common observation among CTD patients is an increased risk of cardiovascular disease (CVD). In SLE, the relative risk for myocardial infarction is increased across all age groups, but up to 52-fold in younger patients. Vitamin D deficiency is a proposed risk factor for the development of CVD in the general population. In SLE, an association between aortic stiffness and vitamin D deficiency has been demonstrated that may be mediated via increased disease activity. Ravenell et al. also found that lower vitamin D was associated with increased carotid plaque, although in this study it was also associated with reduced disease activity. The association between vitamin D and traditional CVD risk factors (e.g. hypertension, hyperlipidaemia, adiposity) is less clear. Some groups have demonstrated an association while others have not. There are currently no published interventional studies to demonstrate that vitamin D can improve cardiovascular outcomes in CTDs. However, in the general population, a meta-analysis of 22 trials demonstrated a significant reduction in all-cause mortality but only a trend towards reduced cardiovascular mortality. Similarly, the Women’s Health Initiative interventional study of 36,282 post-menopausal women found that vitamin D supplementation did not affect CVD risk. This study may have significantly underestimated the effect of vitamin D supplementation, as the control group was allowed personal supplementation (600 IU/day) that was greater than the intervention dose (400 IU/day). Several ongoing trials in the general population are under way and should help to resolve this question in due course.

Metabolic syndrome

The metabolic syndrome is present in ~40% of SLE patients early after diagnosis. Metabolic syndrome is an important risk factor for CVD and is associated with cumulative organ damage. A small study of non-diabetic SLE patients found that low vitamin D was associated with increased insulin resistance and a trend towards increased metabolic syndrome, independent of BMI. In the general population, increases in serum 25(OH)D were associated with significantly reduced risks of developing metabolic syndrome over 12 months.

Fatigue

Fatigue is common in SLE (~80% of patients), often in association with poor sleep, anxiety and depression. In an open-label study of 80 SLE patients there was a significant correlation between the change in serum 25(OH)D and the change in fatigue score over a 2 year period. Further encouraging findings were seen in a randomized controlled trial of cholecalciferol in juvenile-onset SLE by Lima et al. In this study, there was also a significant reduction in fatigue at 24 weeks.

Which patients should be tested?

For the rheumatologist, there are few guidelines to advise which patients should be tested for vitamin D deficiency. The National Osteoporosis Society (NOS) advocates measurement in patients with bone disease (in whom vitamin D may be a treatment or in whom it should be corrected prior to other treatments) or patients with musculoskeletal symptoms that may be due to vitamin D deficiency. However, the NOS does not recommend screening asymptomatic individuals, even if they are at a high risk of vitamin D deficiency. In contrast, the Endocrine Society (a US-based organization) recommends screening a number of patient groups, including those with chronic kidney disease, hepatic failure, obesity and African American and Hispanic populations. Therefore an SLE/CTD population may include many patients in which screening for vitamin D deficiency is appropriate on the basis of assessment of bone health alone.

How much vitamin D is enough?

There is currently no consensus regarding the optimum vitamin D treatment regime. The Institute of Medicine...
has focused only on dietary intake of vitamin D in the general population and recommends a conservative intake of ~600 IU/day [94]. The Endocrine Society suggests a higher daily intake for at-risk individuals, aiming for 1500–2000 IU/day, with an upper limit of 10 000 IU [93]. In the UK, the NOS recommends treatment of deficiency [25(OH)D < 30 nmol/l] with a loading dose of up to 300 000 IU followed by a maintenance dose of ~800–2000 IU/day after a period of 1 month [92]. There are no guidelines relating specifically to CTDs, either in terms of target vitamin D levels or recommended regimes. Our current practise is to focus on bone protection and follow the NOS recommendations, aiming for a target concentration of ~30 ng/ml (75 nmol/l). Any additional effects of vitamin D beyond bone protection are currently theoretical and there is no convincing evidence to encourage a move away from or to enhance current guidelines, either in the scope of screening, the dose regimes or the ideal target vitamin D concentration.

**Vitamin D toxicity**

Vitamin D therapy is usually well-tolerated and vitamin D toxicity is rare. It has been proposed that chronic consumption of ~40 000 IU/day and serum levels in excess of 80 ng/ml (200 nmol/l) are required before toxicity occurs [95]. Similarly, drug–vitamin D interactions are uncommon, but hypercalcaemia may occur when vitamin D is administered concurrently with calcium supplements and thiazide diuretics [96].

**Summary and future areas of research**

There remains considerable interest in the potential use of vitamin D as an adjunct in the treatment of CTDs. Although vitamin D deficiency is common across the CTDs, in observational studies there are numerous factors that confound the association between 25(OH)D and the presence of autoimmune disease. Furthermore, vitamin D levels vary considerably over time and many studies only measure 25(OH)D at a single time point. Mendelian randomization studies of vitamin D pathway polymorphisms may help to identify whether vitamin D deficiency predisposes individuals to developing autoimmune disease.

While experimental studies have demonstrated that 25(OH)D and 1,25(OH)2D3 are anti-inflammatory and immunoregulatory across a number of immune pathways, the results from clinical studies have been inconclusive. There is also a lack of well-designed interventional studies both in SLE and in other CTDs. Many of the studies have used relatively low doses of vitamin D and/or may not have achieved sufficient changes in 25(OH)D levels. It is likely that the optimum serum 25(OH)D level is different for individual patients, thus a personalized approach is likely to be needed. However, the absence of positive results may also point towards a more passive role for vitamin D in autoimmunity, with serum 25(OH)D levels acting as a negative acute phase reactant and thus reflecting the presence of systemic inflammation and general poor health rather than being causative. Well-designed trials are now needed in order to define the utility, if any, of vitamin D to influence or modify the primary disease or its co-morbidities beyond its role in bone health.

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**Supplementary data**

Supplementary data are available at Rheumatology Online.

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Vitamin D in CTDs


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