

# Vitamin D in systemic lupus erythematosus: potential beyond bone health

Vitamin D deficiency is becoming increasingly recognized as playing an important role in autoimmune diseases. The prevalence of vitamin D deficiency in systemic lupus erythematosus is likely to be due to many different factors, including renal disease, sunlight avoidance and disease activity itself. Beyond its role in bone metabolism, vitamin D is an important immunomodulatory agent and may have roles in the pathogenesis of the disease, in addition to the severity of lupus. Musculoskeletal pain, fatigue and depression are common in systemic lupus erythematosus, and vitamin D deficiency is implicated in these conditions. Cardiovascular disease is an important cause of premature mortality in systemic lupus erythematosus. Low-serum vitamin D is associated with increased cardiovascular disease and may provide a potential link between cardiovascular disease and lupus.

**KEYWORDS:** autoimmunity ■ calcitriol ■ cardiovascular disease ■ musculoskeletal pain ■ SLE ■ systemic lupus erythematosus ■ vitamin D

Systemic lupus erythematosus (SLE) is a systemic inflammatory autoimmune disease, predominantly affecting women. It is associated with the formation of a wide range of autoantibodies. Although the overall survival of patients with lupus has improved over recent decades, cardiovascular disease, infection and severe disease activity are still major causes of mortality [1]. Development of further therapies and management strategies for lupus is somewhat limited by an incomplete understanding of the pathogenesis of the disease. The development of SLE is the result of a combination of genetic and environmental factors, as confirmed in recent large collaborative studies [2-5].

Recently there has been interest in the potential role for vitamin D in both the pathogenesis and clinical manifestations of lupus. Vitamin D deficiency is prevalent in SLE, and has potential roles in immune dysregulation, muscular weakness, fatigue and the development of cardiovascular disease (CVD). Although osteoporosis is an important co-morbidity in SLE, a detailed review of the effect of vitamin D on bone health is beyond the scope of this article; in this review we will focus our discussion on the potential role of vitamin D beyond bone health and consider its role in pathogenesis (immune dysfunction), clinical manifestations (musculoskeletal function) and outcomes (CVD) of SLE.

## Vitamin D physiology

Vitamin D is a seco-steroid derived from 7-dehydrocholesterol [6]. Similar to other steroid hormones, it binds to a nuclear receptor, exerting

its action by promoting or inhibiting gene transcription. Unlike other vitamins, which are trace elements obtained from the diet, vitamin D is primarily synthesized in the human body and is thus better described as a hormone. In addition to its genomic action, the active form of vitamin D can bind to a membrane-bound receptor. This promotes calcium influx into the cell, although the physiological importance of this is unknown. It is most likely that an interaction of genomic and nongenomic mechanisms mediate the effects of vitamin D at the cellular level [7].

In the skin, UV light catalyses the conversion of cholesterol derivatives into pre-vitamin D, which is converted to vitamin D<sub>3</sub> without the need for further UV radiation [8]. This remains inactive until it is hydroxylated, initially in the liver to 25(OH)D, then again in the kidney to 1,25(OH)<sub>2</sub>D. 25(OH)D is effectively biologically inactive. Vitamin D<sub>2</sub> (ergocalciferol) is obtained from plant sources and is the form found in most over-the-counter and prescribed vitamin D preparations. Both 25(OH)D and 1,25(OH)<sub>2</sub>D can be measured in the serum by either immunoassay, or preferably high-performance liquid chromatography (HPLC) [9]. When interpreting laboratory values, it is important to consider the technique used, as sensitivity and the normal range can vary significantly. What is clear, however, is that 1,25(OH)<sub>2</sub>D has a short half-life (4 h compared to 3 weeks for 25[OH]D) [10], which means that 25(OH)D provides a far superior estimate of body vitamin D stores.

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As vitamin D synthesis requires sunlight, serum 25(OH)D shows marked seasonal variation [11]. In a study of 90 women aged 20–40 years old in Boston (MA, USA), serum 25(OH)D levels were significantly higher in summer compared with winter [12]. Between February and March, the median serum concentration was 60 nmol/ml (24 ng/ml), compared with 85.4 nmol/ml (34.2 ng/ml) between June and July. Importantly, it is not known whether the peak vitamin D trough level or seasonal average is most useful [13]. Attention also needs to be directed to the method used to measure serum vitamin D. Radioimmunoassays used in the early 1990s were notoriously inaccurate, with wide variability in values obtained. More recently, HPLC and liquid chromatography mass spectrometry have significantly improved the routine measurement of 25(OH)D in serum [14].

#### Classical action of vitamin D: calcium homeostasis

Vitamin D was first identified by its ability to prevent the onset of rickets in animal models. Even now after many other roles have been identified, vitamin D remains synonymous with bone metabolism. Along with parathyroid hormone (PTH), the primary function of vitamin D is to maintain calcium homeostasis [15]. In particular, vitamin D is important in promotion of intestinal calcium absorption via increased expression of  $\text{Ca}^{2+}/\text{H}^{+}$  ATPase in the intestinal enterocyte. [16]. The action of vitamin D on bone mineralization is more complex and beyond the scope of this review. Briefly, the overall effect of vitamin D is to suppress PTH release from the parathyroid glands, and thus prevent PTH-driven dissolution of bone due to increased osteoclast activity [8]. Therefore, significant vitamin D deficiency results in reduced mineralization of bone, manifesting as rickets in children and osteomalacia in adults. The action of vitamin D on the parathyroid gland provides an excellent example of the importance of local regulation of vitamin D concentrations. Physiological concentrations of 25(OH)D suppress PTH release. By contrast, pharmacological concentrations of  $1,25(\text{OH})_2\text{D}_3$  are required. The action of 25(OH)D is dependant on formation of  $1,25(\text{OH})_2\text{D}_3$  by  $1-\alpha$ -hydroxylase within the cell [17]. Circulating active  $1,25(\text{OH})_2\text{D}_3$  may therefore be biologically less important than 25(OH)D, and may have different functions from those synthesized locally.

More recently, vitamin D receptors have also been identified on a wide range of cells, including macrophages, pancreatic cells, somatic cells

and the vasculature. This has led to suggestions that vitamin D may play an important role in infection and autoimmunity, diabetes mellitus, malignancy, angiogenesis and cardiovascular disease [18].

#### Nonclassical action of vitamin D: beyond calcium

It has been increasingly recognized that vitamin D has biological action in around 30 cell systems [7]. Vitamin D has important antiproliferative effects. In colonic enterocytes, there are reduced binding sites for vitamin D in tumor cells compared with healthy cells. Vitamin D is able to arrest cell growth and alter the expression of proto-oncogenes and tumor suppressor genes [19]. Further, in hyperproliferative skin diseases such as psoriasis, vitamin D therapy has well-recognized benefits. Inhibition of proliferation of keratinocytes by vitamin D has been demonstrated *in vitro*, and this observation provides rationale for the therapeutic use of vitamin D analogs in psoriasis [20]. Vitamin D receptors have also been identified in pancreatic  $\beta$  cells. Insulin secretion is promoted by vitamin D and deficiency of vitamin D is associated with glucose intolerance and risk of developing Type 2 diabetes mellitus [21,22].

#### Prevalence of vitamin D deficiency in SLE

There is little doubt that vitamin D deficiency is common in SLE. This observation is not, however, specific to lupus, and occurs in other inflammatory disorders, including rheumatoid arthritis, but not osteoarthritis [23]. A small observational study of 25 patients with SLE showed deficiency ( $<50$  nmol/l, 20 ng/ml) in 14 (56%) patients, although this was not significantly different to results seen in fibromyalgia [24]. A larger study of 92 predominantly Caucasian SLE patients found that 15% had 25(OH)D of less than 10 ng/ml and 75% below 30 ng/ml [25]. This suggests that only one in ten lupus patients have sufficient levels of vitamin D. Elevated PTH was associated with vitamin D deficiency ( $<10$  ng/ml), but not insufficiency ( $<30$  ng/ml). This is an important observation; if vitamin D is above 10 ng/ml, serum PTH tends to remain within the normal reference range. Below this point, it may be difficult to differentiate the effects of low vitamin D from those of raised PTH. It could be argued that the observed deficiency may be due to steroids and other treatments, rather than SLE disease itself. In a case–control study of 123 recently diagnosed SLE patients, vitamin D levels were also significantly lower than controls [26].

The relationship between vitamin D deficiency and disease activity is controversial. The cross-sectional study conducted by Ruiz-Irastorza *et al.*, showed no association between 25(OH)D and activity, as measured by the SLE disease activity index (SLEDAI) in a multiple regression model [25]. In this study, however, 53% of patients had a SLEDAI of 0, potentially underestimating any association with vitamin D. In a smaller study, serum 25(OH)D was lower in the group of patients with highest activity (SLEDAI) compared with those with lower activity and non-SLE controls [27]. In a regression analysis, disease activity was associated with lower 25(OH)D and increased cytokines, especially TNF- $\alpha$ .

Vitamin D deficiencies in SLE patients can be attributed to several causes. As discussed above, vitamin D is principally obtained via mechanisms requiring UV radiation from sunlight [28]. An estimated 57–73% of patients with SLE are photosensitive. This increases to up to 90% in the context of subacute cutaneous lupus [29]. High-factor UV sunblock used to reduce photosensitivity inhibits cutaneous vitamin D synthesis. In patients with biopsy-proven cutaneous lupus, sun avoidance and daily sunblock use is associated with significantly reduced serum 25(OH)D [30]. Unsurprisingly, photosensitivity increases the risk of vitamin D deficiency in SLE by approximately threefold [25].

Skin pigmentation also reduces cutaneous synthesis. In women of child-bearing age, those of African–American origin had a significantly increased prevalence of vitamin D deficiency compared with their Caucasian counterparts [12,31]. Lupus is more common in Afro–Caribbean and South Asian patients [32,33]. Kamen *et al.* showed that deficiency was so prevalent in Afro–Caribbean subjects that there was little difference between SLE patients and controls [26]. Additional cultural factors such as long-sleeved clothing and head coverage may further limit sun exposure. Therefore, ethnicity and cultural factors may partially explain vitamin D deficiency in SLE. Importantly it may also provide a mechanistic link between the ethnic variance in disease prevalence and severity that has been widely reported [32,33]. Insufficient sunlight exposure is, however, unlikely to completely explain all of the observed deficiency in SLE. Other suggested predisposing factors include chronic kidney disease (reduced 1- $\alpha$  hydroxylation), smoking [26] and male gender [25]. The development of antivitamin D antibodies was recently proposed as a potential mechanism, however these were only present in

4% of 171 patients with SLE, which will be too few to satisfactorily explain the prevalence of deficiency [34].

## Vitamin D in SLE pathogenesis

### ■ Immunopathogenesis of SLE

Deficiency of vitamin D has been associated with increased susceptibility to many autoimmune diseases including Type I diabetes mellitus, multiple sclerosis (MS), rheumatoid arthritis and inflammatory bowel disease (Box 1). Interest in a role for vitamin D followed the observation that there was an association between the seasonality of vitamin D and flares of MS. Further, higher serum 25(OH)D was associated with decreased incidence of MS [35]. Even more convincingly, dietary supplementation with vitamin D in infants reduced the chance of developing Type I diabetes mellitus 30 years later [36].

In common with other autoimmune diseases, lupus results from loss of the lymphocyte tolerance and regulatory mechanisms, leading to activation of autoreactive T and B cells [37]. Activation occurs following monocyte-derived antigen presenting cells (APCs) displaying self-antigen (or antigen sufficiently similar to self) to self-reactive CD4<sup>+</sup> T cells, which were ineffectively deleted during early development. These active cells are also dysfunctional and proinflammatory, resulting in the clinical manifestations of the disease.

Systemic lupus erythematosus is often described as a disease of defective apoptosis [38,39]. Failure to clear apoptotic debris leads to increased uptake and presentation of altered self-antigens by dendritic cells. This results in activation of autoreactive CD4<sup>+</sup> T-helper cells and B cells, as well as the development of the characteristic autoantibodies against nuclear components. More recently, it has been suggested that in addition to defective phagocytosis, the monocytes in SLE have an abnormal cytokine profile, with excessive production of IL-6 and IL-10 [40,41]. In SLE, there are also decreased numbers of T-regulatory cells (Tregs) [42]. These cells express CD4/CD25 and are important in the downregulation of activated T cells, and

#### Box 1. Autoimmune diseases associated with vitamin D deficiency.

- Type 1 diabetes mellitus
- Multiple sclerosis
- Rheumatoid arthritis
- Inflammatory bowel disease
- Autoimmune thyroiditis
- Systemic lupus erythematosus

in the context of SLE, autoreactive CD4<sup>+</sup> T cells, which facilitate B-cell production of auto-antibodies. Lupus is not, however, only a disease of T cells. B lymphocytes, which are important in T-cell and antigen presenting cell regulation, are also hyperactive in lupus, with increased expression of IL-10 and IL-6 [43].

#### ■ Vitamin D & immune system regulation

Vitamin D receptors (VDRs) are present throughout the cells of the immune system. Antigen presenting cells, and the monocytes from which they are derived, express the VDR [44]. T lymphocytes, fundamental to the autoimmune state, can also be induced to express the VDR. Vitamin D is implicated in immune regulation at different stages throughout the immune response.

Monocytes circulate in the peripheral blood and are precursors of dendritic cells. Activation of monocytes by IFN- $\gamma$  promotes monocytes-T cell interaction. This activation is associated with upregulation of 1- $\alpha$ -hydroxylase in monocytes [45]. In Type 2 diabetes, the monocytes have a proinflammatory profile, with increased expression of TNF- $\alpha$ , IL-1, IL-6 and IL-8, similar to the abnormal profile seen in SLE. 1,25(OH)<sub>2</sub>D<sub>3</sub> downregulates the expression of these cytokines [46]. This suggests firstly, that activation of monocytes is associated with local 1,25(OH)<sub>2</sub>D<sub>3</sub> synthesis, providing autoregulation of the immune process. This would provide supra-physiological local concentrations of the hormone. Secondly, vitamin D may have a role in suppressing monocyte-derived inflammation in SLE-suppressing inflammatory cytokines.

The ability of monocytes to activate T cells is significantly impaired by the presence of 1,25(OH)<sub>2</sub>D. Vitamin D induces differentiation into macrophages, whilst reducing expression of MHC class II and co-stimulatory molecules [47]. Vitamin D inhibits the differentiation of monocytes into dendritic cells. These cells are more effective at activating T cells than monocytes, and thus their reduction has important implications for lymphocyte activation [48]. IL-12 is the dominant lymphocyte-activating cytokine of APCs. Its production, and thus lymphocyte activation, is reduced by 1,25(OH)<sub>2</sub>D [49].

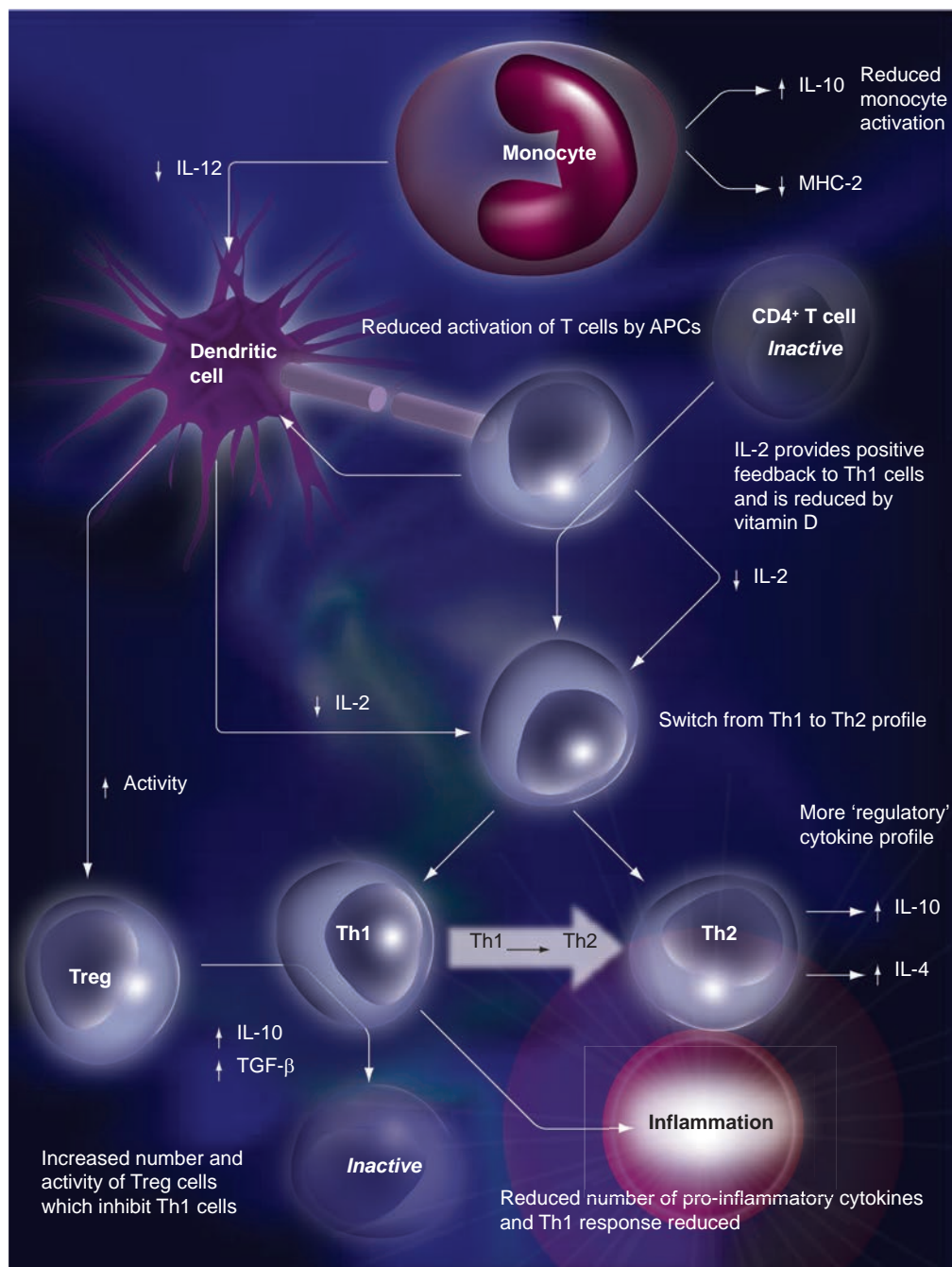
Loss of tolerance mechanisms result in activation of autoreactive T and B lymphocytes in SLE. Tolerogenic dendritic cells, which activate Treg cells, are reduced in SLE, resulting in fewer regulatory cells [50]. Development of these dendritic cells can be induced by biological agents (e.g., anti-CD40L), anti-inflammatory cytokines

(e.g., TGF- $\beta$ ), corticosteroids, immunosuppressant therapies and vitamin D [51]. Vitamin D analogs increase the number of dendritic cells, thus increasing the number of active regulatory lymphocytes. Therefore, in SLE, replacement of vitamin D may increase the observed low numbers of Treg cells, leading to increased immune regulation [51].

The CD4<sup>+</sup> T-lymphocyte response broadly comprises the inflammatory (Th1) and regulatory (Th2) pathway. The Th1 response is characterized by IFN- $\gamma$  and IL-2, whilst in the Th2 response, IL-4 predominates. CD4<sup>+</sup> lymphocytes express the VDR, and this expression is increased fivefold when the cell is activated [52]. *In vitro*, 1,25(OH)<sub>2</sub>D appears to indirectly shift the balance towards a Th2 response by inhibiting proliferation of Th1 lymphocytes and reducing their dominant cytokines, IL-2 and IFN- $\gamma$ . This results in migration towards the Th2 phenotype. IFN- $\gamma$  acts as positive feedback from Th1 cells to APCs, whilst IL-2 provides positive feedback to the Th1 lymphocytes themselves. Both reduction of IL-12 and increase in IL-10 production by APCs under the influence of vitamin D favors the Th2 response (see FIGURE 1). In SLE, there seems to be an increased Th1:Th2 ratio, especially in the context of lupus nephritis [53]. Vitamin D, therefore, has the potential to reverse this ratio, thus reducing the inflammatory response.

The effect of 1,25(OH)<sub>2</sub>D on IL-4 production, however, remains controversial [44], although this may be because the vitamin D-induced Th1/Th2 switch may be subtly different *in vitro* and *in vivo*. Importantly, in the context of SLE, vitamin D reduces autoantibody production by peripheral blood mononuclear cells obtained from lupus patients [54]. This may be particularly pertinent in lupus nephritis where anti-ds-DNA antibodies may be directly pathogenic [55]. The association between 25(OH)D status and the presence/absence of autoantibodies has not been fully determined. Vitamin D can also modify the CD4<sup>+</sup> T-cell response by increasing activity of the Treg cell. These cells secrete the inhibitory cytokines IL-10 and TGF- $\beta$ , which inhibit antigen-specific T-cell activation. Therefore, vitamin D may suppress inflammation in SLE both by increasing the activation of Treg cells by tolerogenic dendritic cells, and by increasing the activity of Treg cells. The effect of vitamin D on Th17 cells remains unclear.

Therefore, one might postulate that vitamin D seems to reduce T-cell activation, thus resulting in impaired immune response to pathogens. It should be remembered, however, that vitamin D appears to regulate rather



**Figure 1. Roles of vitamin D in immune system regulation.** Vitamin D regulates cytokine production from monocytes, dendritic cells and T cells. The overall effect is to switch the T-cell response from the inflammatory Th1 response to the more regulatory Th2 response. Therefore, vitamin D has the potential to reduce inflammation. The arrows show the effect of vitamin D on production of the cytokine.

↓: Decreased; ↑: Increased; APC: Antigen presenting cell; IL: Interleukin; Treg: Regulatory T cell; IFN: Interferon.

than suppress the adaptive immune system whilst stimulating the innate immune system by increasing monocytes chemotaxis and phagocytosis [50].

B-cell hyperactivity in SLE and failure to remove autoreactive B cells results in the production of autoantibodies and propagation of the

inflammatory response. B cells that express the VDR and  $1,25(\text{OH})_2\text{D}_3$  can inhibit the proliferation of these cells, and even induce apoptosis [56]. Further to this, vitamin D inhibits plasma-cell differentiation and secretion of immunoglobulins, providing a possible mechanism for reduced autoantibody production.

Whilst of interest, the pathways above are somewhat hypothetical and experimental and clinical confirmation is required. In mouse models of lupus, results of treatment with vitamin D are conflicting. The MRL/lpr mouse spontaneously develops symptoms of SLE. Following treatment with a VDR-ligand, the mice had fewer cutaneous manifestations, reduced proteinuria and greater longevity [57]. Conversely, in NZBxWF1 mice,  $1,25(\text{OH})_2\text{D}$  exacerbated lupus nephritis on biopsy [58]. This provides an important reminder about the relative weaknesses of animal models, and that they may not correlate directly to SLE in humans. Therefore, vitamin D therapy has the potential to realign the immune system in SLE by reducing T-cell activation, increasing regulation of active T cells, reducing B-cell activity and modulating the cytokine profile away from an inflammatory response. Treatment with  $25(\text{OH})\text{D}$  could result in increased  $1,25(\text{OH})_2\text{D}_3$  by monocytes, resulting in increased local concentrations in a paracrine manner.

#### **Vitamin D & musculoskeletal function, fatigue & depression**

Depression, fatigue and generalized muscle aches are well-recognized nonspecific symptoms of SLE. They occur even in mild disease and may be under-recognized by the nonspecialist, as they lie outside the ACR criteria. Up to 50% of patients with lupus have symptoms of depression [59] and the majority of patients complain of fatigue, which can be extremely debilitating [60,61].

Osteomalacia is characteristically associated with proximal myopathy [62]. It has recently been shown that vitamin D deficiency can adversely affect muscle function at levels that are not severe enough to cause true osteomalacia. In the elderly, vitamin D deficiency is independently associated with impaired muscular function, as measured by the ability to perform daily activities (aggregate functional performance time [AFPT]) and postural stability [63]. Similarly, in women with postmenopausal osteoporosis, there is increased body sway and limitations of activities of everyday life in patients with low levels of vitamin D [64]. Muscle strength and muscle mass have been measured in a Dutch cohort. Vitamin D deficiency and raised serum PTH were independently associated with loss of muscle function and muscle mass (sarcopenia) in this aging population [65]. Following a long-bone fracture,  $25(\text{OH})\text{D}$  is independently associated with muscle strength, as measured over 12 months out-patient follow-up [66]. The VDR is expressed in skeletal muscle

and appears to decline with age [67]. This may explain the above observations and should prompt investigation of the muscle VDR in active lupus.

In SLE, patients with a serum  $25(\text{OH})\text{D}$  level below the lower limit of the normal range for the assay used have been shown to have impaired function and experience greater pain compared with those with higher vitamin D levels. There was a statistically significant difference using the global visual analog scale, both alone and when combined with the modified health assessment questionnaire and the fatigue global visual analog scale [68]. Vitamin D deficiency is also associated with fatigue in SLE. Borba *et al.* noted that in patients with  $25(\text{OH})\text{D}$  below 10 ng/ml there was a trend towards patients having more fatigue [27].

A large case-control study in the general population shows 15% lower  $25(\text{OH})\text{D}$  levels in patients with major or minor depression compared with healthy controls. Severity of depression was associated with serum PTH and inversely with vitamin D [69]. Supplementation with vitamin D also improves symptoms of depression in patients with obesity [70]. There are no data in SLE patients examining mood and vitamin D levels.

#### **Cardiovascular risk**

SLE is associated with a significantly increased risk of developing cardiovascular disease, even after traditional risk factors are taken into account [71]. Whilst there is an increased prevalence of hypertension and diabetes in lupus, a discrepancy exists between the estimated cardiovascular risk and the much greater observed incidence [72]. The increased risk of developing CVD in SLE, compared with the general population, is particularly pronounced in younger patients. Whilst the overall relative risk is estimated to be 5- to 6-times, in women aged 35-44 years this may be as great as 52-fold [73]. The observed fatal and nonfatal cardiac events at this earlier age occur on a background of increased subclinical atherosclerosis. The presence of carotid artery plaque is a well-recognized predictor of future cardiovascular events [74]. In SLE, the presence of carotid plaque is associated with disease activity and duration [75]. Indeed, the progression of plaque and carotid intima-media thickness (IMT; a noninvasive predictor of CVD) is accelerated in SLE after adjustment for traditional risk factors [76]. Endothelial dysfunction, as measured by flow-mediated dilatation, is common in SLE [77,78] and may predict future cardiovascular events in lupus patients [79].

There is much speculation about the origins of this premature cardiovascular disease. Systemic inflammation has shown to be independently associated with atherosclerosis through many different mechanisms [80]. It is proposed that the combination of inflammation and pathological immune responses, insulin resistance, dyslipidemia and other yet unidentified factors all contribute to the increased CVD risk (Box 2). Inflammatory cytokines, including TNF, IFN, IL-1 and IL-12 have recently also been implicated [81]. As discussed above, vitamin D deficiency may contribute to this proinflammatory state. By this mechanism and others discussed below, vitamin D deficiency may accelerate atherosclerosis in both SLE patients and in the general population.

### ■ Vitamin D & CVD risk

In the 1970s, seasonal variation was observed in the incidence of cardiac events; these events were much more common in the winter months [82,83]. In addition, CVD is more common in northern latitudes [84]. Ecologically, therefore, an intriguing hypothesis would involve vitamin D since, as we have already noted, serum vitamin D is lower in winter and in those living further from the equator. At present, no studies have examined the relationship between vitamin D deficiency and CVD in SLE. However, recent evidence suggests that vitamin D deficiency is associated with an increased incidence of cardiovascular disease in the general population, particularly in those at high risk for CVD.

The Framingham Offspring Cohort consists of 5124 subjects followed prospectively for CVD risk and outcomes. Wang *et al.* measured serum 25(OH)D<sub>3</sub> in 1972 consecutive patients without history of cardiovascular disease [85]. Over a mean 5.4-years follow-up, the 5-year rate of fatal and nonfatal cardiovascular events was 8.9% (95% CI: 5.5–12.2) in subjects with serum 25(OH)D<sub>3</sub> less than 15 ng/ml, but only 4.4% (95% CI: 2.8–5.9) in those with 25(OH)D<sub>3</sub> above 15 ng/ml. The influence of vitamin D was augmented in those with hypertension (14.2 vs 5.8%) and persists after adjustment for classical risk factors. Intriguingly, the association appears to be nonlinear. Low vitamin D levels were associated with an increased risk of CVD. There also appeared to be a slight increase in risk with higher vitamin D levels, although the power to detect this was limited, owing to the low prevalence of higher vitamin D levels in the population [85].

Other smaller studies have found similar results. The risk of myocardial infarction in men is approximately doubled for serum 25(OH)D<sub>3</sub>

### Box 2. Risk factors for increased prevalence of cardiovascular disease in systemic lupus erythematosus.

#### **Increased traditional risk factors**

- Hypertension
- Type 2 diabetes mellitus
- Hypercholesterolemia
- Smoking

#### **Potential novel risk factors**

- Oxidized low-density lipoprotein
- C-reactive protein
- Homocysteinemia
- Anticardiolipin antibodies
- Endothelial dysfunction
- Type I interferon
- Chronic kidney disease

below 15 ng/ml compared with above 30 ng/ml, with a linear relationship between vitamin D and MI events [86]. In high-risk patients referred for coronary angiography, an association was also seen between vitamin D deficiency and both cardiovascular and all-cause mortality [87]. Other smaller studies have shown smaller or nonsignificant associations with vitamin D and vascular disease, however these studies likely lack statistical power to fully assess this relationship in detail [88–90].

Vitamin D deficiency as a novel independent risk-factor for cardiovascular disease does, however, cluster with other more traditional risk factors. In the above observational studies, subjects with low serum 25(OH)D were more likely to be overweight, diabetic, hypertensive or smoke, and have an unfavourable lipid profile [86,87]. Therefore, a direct causal link is difficult to confirm. However, an alternative and equally plausible hypothesis is that low vitamin D status may be a marker of overall poor health or a poor, at-risk lifestyle.

If vitamin D itself is protective to the vasculature, the observational study by Dobnig *et al.* may also, however, provide some clues to the mechanisms at play. Vitamin D deficiency was significantly associated with markers of inflammation (C-reactive protein and IL-6), increased expression of vascular adhesion molecules and markers of oxidative stress [87].

### ■ Potential mechanisms: hypertension

As previously discussed, SLE is associated with an increased prevalence of hypertension [73]. Vitamin D deficiency may also be associated with hypertension, providing a link between the two conditions, although the evidence is much less compelling than that presented above. Three large

prospective cohort studies showed no increased risk of developing hypertension with low vitamin D intake; relative risk (95% CI) for low versus high intake was 0.98 (0.93, 1.04), 1.13 (0.99, 1.29) and 1.03 (0.93, 1.15) [91]. Unlike the previous studies, however, vitamin D was estimated using semiquantitative dietary questionnaires, and thus comparison with actual serum vitamin D measurement is questionable [12]. If serum 25(OH)D is used instead, an inverse relationship with systolic blood pressure (BP) is seen. In 7699 patients not previously diagnosed with hypertension, the age-associated rise in systolic BP was attenuated with higher serum vitamin D. A linear relationship existed between serum 25(OH)D and systolic pressure in Caucasian subjects, although this was no longer significant after adjusting for age [92]. Dietary supplementation with vitamin D in the Women's Health Initiative study (vitamin D 200 IU, twice daily) did not affect systolic BP, or the risk of developing hypertension over 7 years in 36,282 postmenopausal women [93]. Analysis of cardiovascular events in the same cohort again found no benefit of oral vitamin D supplementation. However, these studies do not exclude a potential benefit of vitamin D treatment in CVD, as current estimates suggest doses of 800–1000 IU/day are probably required for maintaining adequate vitamin D status [94].

#### ■ Potential mechanisms: vascular reactivity

Recent animal studies give some clues about vascular health and vitamin D status. In the spontaneously hypertensive rat there is impairment of endothelium-dependent vascular relaxation mediated by acetylcholine. After oral vitamin D treatment, the systolic BP and basal perfusion pressure of the mesenteric bed returned to normal [95]. *In vitro*, treatment of mesenteric rings from spontaneously hypertensive rat with vitamin D changes endothelium-dependent relaxation from nitric oxide-mediated to endothelium-derived hyperpolarizing factor-mediated, occurring in nonhypertensive rats, possibly by recruitment of silent  $K^+$  channels [96]. Nitric oxide is an important mediator of vascular relaxation. TNF causes reduced expression of endothelial nitric oxide synthase (eNOS) in human endothelial cells by destabilizing mRNA [97,98]. Advanced glycation end products, which are common in diabetes, also down-regulate eNOS, resulting in reduced synthesis of NO in cultured human endothelial cells. The addition of 1,25(OH)<sub>2</sub>D<sub>3</sub> to the culture medium protects eNOS from this insult [99].

Brachial artery flow-mediated dilatation is impaired in both SLE and diabetes. In diabetes, a single intramuscular dose of vitamin D (100,000 IU), significantly improved endothelium-dependent flow-mediated dilatation in a blinded, placebo controlled trial [100]. It remains to be seen whether endothelial dysfunction can be reversed in lupus in a similar manner.

#### ■ Potential mechanisms: atherogenesis

In discussing the potential role of vitamin D deficiency and predisposition to CVD, it is important to recognize that atherogenesis is predominantly an inflammatory process. Briefly, plaque development begins with attraction and binding of T lymphocytes and macrophages to the endothelium. These endothelial leukocyte adhesion molecules are upregulated by inflammatory cytokines. The migration of these cells into the intimal layer is again regulated by inflammatory molecules and is associated with smooth muscle cell hypertrophy and lumen narrowing. The formation of foam cells from macrophages and oxidized low-density lipoprotein, requires inflammation to attract low-density lipoprotein into the vascular wall where it is oxidized by reactive oxygen species [101].

In patients with Type 2 diabetes mellitus, vitamin D deficiency is independently associated with increased carotid IMT [102]. Given the established link between IMT and CVD in lupus, vitamin D deficiency may form part of a common pathological process in both conditions.

Cellular activation of 25(OH) to 1,25(OH)<sub>2</sub>D requires expression of the cytoplasmic enzyme 1- $\alpha$ -OHase. In renal tubular cells, this enzyme is regulated by both 1,25(OH)<sub>2</sub>D<sub>3</sub> and calcium [103]. Both human vascular endothelial cells and human smooth muscle cells express 1- $\alpha$ -OHase, which is increased by TNF and lipopolysaccharide [104,105]. Indeed both of these cell types also express the receptor [104,106], suggesting a micro-endocrine environment within the vasculature where cells are able to both activate vitamin D and respond to it.

The effects of 1,25(OH)<sub>2</sub>D on cell growth, however, remain controversial. Addition of 25(OH)D and 1,25(OH)<sub>2</sub>D to endothelial cells in culture arrests growth [104], but may either increase or decrease smooth muscle cell proliferation [105,107]. Active SLE results in upregulation of leukocyte adhesion molecules VCAM and ICAM [108], a process that is also driven by TNF [104,109]. Both 25(OH)D and 1,25(OH)<sub>2</sub>D also increase leukocyte migration via VCAM–ICAM or independent



mechanisms, although the implications of this are far from clear [103]. We have demonstrated increased expression of 1- $\alpha$ OHase in the endothelium, smooth muscle cells and macrophages in atherosclerotic lesions when compared to disease-free vessels [REYNOLDS J, UNIVERSITY OF MANCHESTER, UK, UNPUBLISHED DATA].

### Future perspective

Vitamin D has come a long way since its described role in treatment and prevention of osteomalacia and rickets. The VDR is present in many organ systems and its actions extend far beyond bone metabolism. The association between vitamin D deficiency and autoimmunity is an important one, as it may herald clues to the development of autoimmunity. A deeper knowledge of the mechanisms at play holds the potential to prevent, if not reverse, immune system dysfunction. Cardiovascular disease is important in SLE and is often under-recognized. As patients with lupus live longer due to improved immunosuppressant therapy, early-onset CVD has emerged as a significant threat in our patients. In SLE, many novel risk factors increase the cardiovascular risk, limiting the utility of traditional risk calculations. In large population studies, vitamin D deficiency is associated with increased CVD. In experimental models, vitamin D appears to reduce endothelial dysfunction and may be involved in preventing development of atherosclerotic plaque. The precise role of vitamin D is poorly understood and much work needs to be done to further understand the mechanisms at work.

Central to these proposals is the idea that vitamin D deficiency is itself pathogenic. The large scale observational studies show an association between deficiency and disease but the number of interventional studies is limited to a few small trials, none of which are in patients with SLE. It is entirely possible that vitamin D is little more than an indicator of general health status, which is reduced in chronic inflammation or in those with pre-existing subclinical vascular disease. Since vitamin D is modifiable, it is of significant interest as a novel biomarker with prognostic significance. The results of the small trials in the general population are compelling, and achieve statistical significance despite small numbers. Larger studies are required for confirmation.

If vitamin D is to be used as a therapeutic agent, then questions still need to be addressed regarding the definition of vitamin D deficiency. There is widespread variation in serum 25(OH)D between populations. This is attributed to environmental exposure (latitude and season), ethnic variation, sunlight exposure/avoidance and other factors, including obesity, renal disease, smoking and co-morbid diseases. This is further complicated by variability in the biochemical methods used to obtain serum 25(OH)D values. For this second concern, there needs to be widespread consensus within the scientific community and adoption of uniform standards to ensure minimal variability between the different methods. Previous definitions of vitamin D deficiency are based on PTH suppression. Below 30 ng/ml of 25(OH)D,

### Executive summary

#### **Vitamin D deficiency in systemic lupus erythematosus**

- Vitamin D deficiency is common in systemic lupus erythematosus (SLE) and appears to correlate with disease activity.
- The cause of the vitamin D deficiency in lupus is not clear, but renal disease, sunlight avoidance and dark skin pigmentation are important.

#### **Vitamin D has a role in modulation of the immune system**

- Activation of Th1-type CD4<sup>+</sup> lymphocytes is reduced by vitamin D via inhibitory effects on the antigen presenting cells, reduced inflammatory cytokine production by activated T cells and increased number and activity of regulatory T cells.
- Vitamin D reduces B-cell activation and antibody production.
- In mouse models of lupus, vitamin D may improve disease activity.

#### **Vitamin D deficiency may be responsible for some of the non-organ-specific manifestations of SLE**

- Fatigue, widespread musculoskeletal pain and depression are common in SLE.
- Depression is associated with low serum vitamin D/raised parathyroid hormone and is improved by vitamin D supplementation.
- Skeletal muscle expressed the vitamin D receptors and vitamin D deficiency is independently associated with decreased muscle strength.

#### **Vitamin D deficiency is associated with increased cardiovascular risk**

- Observational evidence shows that vitamin D deficiency is an independent risk factor for cardiovascular disease in the healthy population and in at-risk populations.
- Vitamin D deficiency may be a useful biomarker in assessing cardiovascular risk.
- Vitamin D potentially acts in a micro-endocrine environment within the vasculature, thus having a role in atherogenesis.
- Whether vitamin D deficiency itself is pathogenic remains to be determined, however, supplementation with vitamin D appears to reverse endothelial dysfunction in both patients and animal models.

there is an incremental rise in serum PTH, leading authors to propose levels above 30 ng/ml as a replete vitamin D level. This may be appropriate from the point of view of bone metabolism but there are already suggestions that a single cut point does not unite all vitamin D-related systems. When analyzing studies of vitamin D, it is important to consider this reciprocal relationship and recognize that, at low levels of vitamin D, there are also changes in serum PTH levels. Teasing apart the contribution of low vitamin D from that of raised PTH becomes a challenge, especially as even small increases of PTH (within the normal adult range) may be significant.

Vitamin D deficiency in SLE is likely to be of increasing importance as the full functions of this hormone are identified. The potential

to improve quality-of-life and reduce mortality and morbidity in lupus is extremely attractive, especially if it can be achieved with such a simple intervention. For this to be achieved, however, well conducted trials and prospective studies will be necessary.

#### Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

*No writing assistance was utilized in the production of this manuscript.*

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