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Does vitamin D play a role in autoimmune endocrine disorders? A proof of concept

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Abstract In the last few years, more attention has been given to the "non-calcemic" effect of vitamin D. Several observational studies and meta-analyses demonstrated an association between circulating levels of vitamin D and outcome of many common diseases, including endocrine diseases, chronic diseases, cancer progression, and autoimmune diseases. In particular, cells of the immune system (B cells, T cells, and antigen presenting cells), due to the expression of 1α-hydroxylase (CYP27B1), are able to synthesize the active metabolite of vitamin D, which shows immunomodulatory properties. Moreover, the expression of the vitamin D receptor (VDR) in these cells suggests a local action of vitamin D in the immune response. These findings are supported by the correlation between the polymorphisms of the *VDR* or the *CYP27B1* gene and the pathogenesis of several autoimmune diseases. Currently, the optimal plasma 25-hydroxyvitamin D concentration that is necessary to prevent or treat autoimmune diseases is still under debate. However, experimental studies in humans have suggested beneficial effects of vitamin D supplementation in reducing the severity of disease activity. In this review, we summarize the evidence regarding the role of vitamin D in the pathogenesis of autoimmune endocrine diseases, including type 1 diabetes mellitus, Addison's disease, Hashimoto's thyroiditis, Graves' disease and autoimmune polyendocrine syndromes. Furthermore, we discuss the supplementation with vitamin D to prevent or treat autoimmune diseases.

Keywords Vitamin D · Autoimmunity · Type 1 diabetes mellitus · Addison's disease · Hashimoto's thyroiditis · Graves' disease · Autoimmune polyendocrine syndromes · Environment · Lifestyle

1 Introduction

Vitamin D_3 (cholecalciferol) is a steroid hormone precursor synthesized within skin under the photochemical reaction influenced by ultraviolet B radiation of sunlight. Dietary intake of vitamin D is generally limited to oily fish and eggs. Cholecalciferol is biologically inert and requires two succes-

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sive hydroxylation reactions for activation. The first hydroxylation occurs in the liver to form the 25-hydroxyvitamin D_3 [25(OH)D_3 or calcidiol], which is converted in the kidney to the biologically active compound 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$ or calcitriol] by the 1α-hydroxylase (CYP27B1), an enzyme which is stimulated by parathyroid hormone (PTH) [1]. Calcitriol acts through binding the nuclear vitamin D receptor (VDR) that mediates the transcription of several target genes [2].

The main physiologic role of vitamin D is the regulation of mineral and bone metabolism. Nowadays, the expression of both 1α-hydroxylase and VDR beyond tissues involved in classical endocrine pathway, including colon, breast, pancreas, malignant cells and immune cells, suggests that also other tissues, different from kidney, are able to synthesize the active form of vitamin D [3, 4]. Thus, vitamin D has pleiotropic effects and may act in paracrine or autocrine manner in addition to its endocrine function. This suggests an important impact of vitamin D in the pathogenesis and outcome of many common diseases, including endocrine diseases [5], chronic diseases [6] and cancer progression [7, 8].

Particularly, cells of the immune system (B cells, T cells and antigen presenting cells), due to the expression of 1α-hydroxylase [9], are able to synthesize the active metabolite of vitamin D, which shows immunomodulatory properties similar to locally active cytokines [10]. Moreover, differently to what happens in the kidney, the 1α-hydroxylase presented on macrophages and dendritic cells is not regulated by PTH, calcium and 1,25$(OH)_2D_3$, but is predominantly regulated by interferon (IFN)-γ and lipopolysaccharides [11]. Recently, Hossein-nezhad et al. demonstrated that vitamin D supplementation *in vivo* significantly regulated the expression of 291 genes in white blood cells, which interfere with more than 160 distinct pathways linked to cancer, autoimmune disorders and cardiovascular diseases [12]. However, the pathway associated with immunological responses seems to have a prominent position, demonstrating that vitamin D is an important immune regulator, both to the innate and adaptive immune responses [12].

According to this, several observational studies and meta-analyses demonstrated an association between circulating levels of vitamin D and autoimmune disorders, including type 1 diabetes mellitus, Addison's disease, autoimmune thyroid disease, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, systemic lupus erythematosus [5, 13, 14] and infectious disease [15]. Many observational studies and meta-analyses have demonstrated strong associations between low circulating concentrations of vitamin D and non-skeletal disease and their outcomes [16].

Our review focuses on the reported association between vitamin D status and autoimmune endocrine disease and the role of vitamin D supplementation to reduce autoimmune disease risk by modulating the immune system.

2 Methods: search strategy and selection criteria

Relevant literature was searched in multiple databases, including PubMed/Medline, EMBASE, and the Cochrane Library up to October 2016. The following specific keywords were used alone or in combination for the search: vitamin D, cholecalciferol, calcidiol, calcitriol, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, autoimmunity, type 1 diabetes mellitus, Addison's disease, Hashimoto's thyroiditis, Graves'disease, autoimmune polyendocrine syndromes. Boolean operators (AND, OR, NOT) were also used to increase the sensitivity of the search. Studies in non-English languages, as well as letters to the editor, conference abstracts and editorials, were excluded. Studies were evaluated by title, abstract relevance, and importance and availability of the full text. Additionally, the reference lists of original articles and reviews were cross-referenced to find further eligible articles. All full texts of the included studies were successively screened and discussed by the panel of authors until a general consensus was reached. Manuscripts not focused on the topic were excluded, and the full text of the remaining selected studies was reviewed in this paper.

3 Vitamin D and autoimmune diseases: clinical and basic evidence

Autoimmune diseases are characterized by a loss of immune homeostasis resulting in failure of self-recognition followed by the destruction of body tissue by autoreactive immune cells. A combination of genetic predisposition, epidemiologic risk factors and environmental contributors may lead to the development of autoimmune disease [14, 17]. Among the mechanisms contributing to the development of autoimmunity, one important factor could be represented by insufficient vitamin D levels, as mounting epidemiologic evidence suggests an association between vitamin D deficiency and a higher incidence of autoimmune diseases.

Vitamin D has been found to play a significant role in the function of the immune system, in both innate and adaptive immunity [18]. Indeed, many immune cells express vitamin D receptors. Besides VDR, immune cells also express the 1α-hydroxylase, which converts 25(OH)D into its active form 1,25$(OH)_2D_3$. Thus, all these cells are capable of responding not only to the active vitamin D metabolite but also to its precursors, and possess a mechanism to convert vitamin D [19].

Vitamin D is an important mediator of innate immune responses enhancing the antimicrobial properties of immune cells, such as monocytes and macrophages. The historical link between vitamin D and innate immune function relies on the use of cod liver as treatment for tuberculosis [20]. In fact, 1,25$(OH)_2D_3$ intensifies the antimicrobial peptide activity in

monocytes, neutrophils and other cell lines [21]. In particular, vitamin D has been found to modulate gene expression in response to a *Mycobacterium tuberculosis*' immune challenge. Potential targets for this response include the antibiotic protein cathelicidin that represents a direct transcriptional target for the $1,25(OH)_2D_3$–VDR complex. The vitamin D-mediated induction of cathelicidin has been found to enhance the killing of *Mycobacterium tuberculosis* in monocytes [20]. Beyond cathelicidin, vitamin D stimulates the expression of other potent antimicrobial peptides, such as β defensin 2 [22], which exist in neutrophils, monocytes, natural killer cells and epithelial cells lining the respiratory tract.

These findings may be of particular interest considering that autoimmune responses can be triggered by infectious agents [23] and an association has been found between low levels of vitamin D and many infections [24]. Of note, Epstein Barr virus, one of the most compelling infectious agents, while considering induction of autoimmunity, also shows an association with vitamin D levels, as it leads to a down-regulation of VDR expression [25].

Vitamin D has important effects on both monocytes and dendritic cells (DC) [21]. It inhibits monocyte production of inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-8, IL-12 and tumor necrosis factor (TNF)-α. Additionally, it inhibits DC differentiation and maturation with preservation of an immature phenotype as evidenced by a decreased expression of major histocompatibility complex (MHC) class II molecules, co-stimulatory molecules and IL-12 [1] (Fig. 1). Moreover, vitamin D is also involved in the humoral response indirectly causing suppression of T cell proliferation, resulting in a shifting from a T-helper (Th)1 to a Th2 phenotype [26]. Influencing the phenotype and function of DC, with the consequent inhibition of their differentiation and maturation, the final effect of vitamin D is a reduction of the number of antigen-presenting cells that stimulate T cells, thus favoring T-cell tolerance.

Inhibition of DC differentiation and maturation is particularly important in the context of autoimmunity and the abrogation of self-tolerance. Indeed, antigen presentation to a T cell by a mature DC facilitates an immune response against that antigen, whereas antigen presentation by an immature DC facilitates tolerance. Interestingly, self-antigens are abundant in the normal state from physiologic cell death and turnover; however, presentation of these self-antigens is usually driven by immature DC so that tolerance to self is maintained [1]. In particular, $1,25(OH)_2D_3$ affects T cell maturation with a skewing away from the inflammatory Th17 phenotype. These effects result in a decreased production of inflammatory cytokines such as IL-17 with increased production of anti-inflammatory cytokines such as IL-10; furthermore, vitamin D facilitates the induction of T regulatory cells (Tregs) (Fig. 1). Tregs function to decrease the immune response by regulating the activity of other T cells through a diverse array of mechanisms including secretion of anti-inflammatory cytokines, and consumption of the T-cell growth and survival factor, IL-2 [27]. Tregs may also induce direct cytolysis of target cells and impair the capacity of antigen presenting cells to prime adaptive immune response. Through such mechanisms, vitamin D is thought to modulate cell-mediated immune responses and regulate inflammatory T cell activity. Of note, recent data show that Th17 cells also participate in pregnancy-related pathologies, including recurrent spontaneous abortion and pre-eclampsia, and imbalances between Th1/Tregs/Th17 subsets in both circulation and uterus have been reported [28]. Interestingly, it has been shown that the odds of developing pre-eclampsia and eclampsia may increase by up to 5-fold in women with vitamin D insufficiency [29].

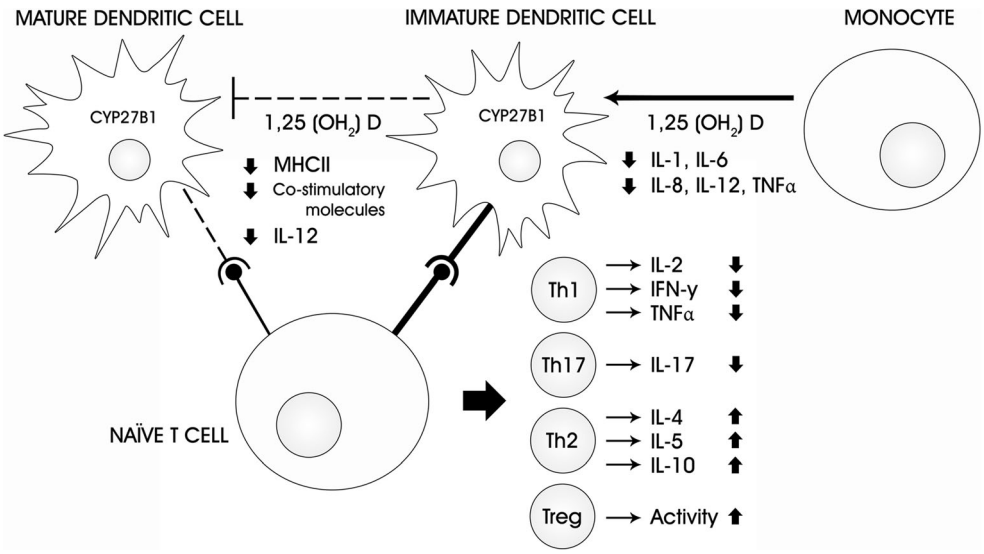

Fig. 1 The immunomodulatory effects of the vitamin D on immune cells. Effects of vitamin D on monocytes and dendritic cells include inhibition of inflammatory cytokine production by monocytes and inhibition of dendritic cell differentiation and maturation, which in turn leads to suppression of T cell proliferation and results in a shift from a T-helper (Th)1 to a Th2 phenotype. Vitamin D affects T cell maturation with a skewing away from the inflammatory Th17 phenotype and facilitates the induction of T regulatory cells (Tregs)

However, also B cells express VDR, and the differentiation into plasma cells and post-switch memory B cells has been found to be inhibited in the presence of $1,25(OH)_2D_3$ [30]. Therefore, vitamin D can also modulate immunoglobulin production and exert direct effects on B cell homeostasis.

In summary, vitamin D activity may enhance the innate immune system and regulate the adaptive immune system, promoting immune tolerance and acting to the decrease the likelihood of developing autoimmune disease.

4 Vitamin D and type 1 diabetes mellitus

Conflicting data exist on a role for the vitamin D system in type 1 diabetes mellitus (T1DM). Although some studies suggest a linkage between polymorphisms of the *VDR*, others failed to do so. A large meta-analysis found an association between BsmI polymorphism and T1DM risk in the Asian population, with a 30% increased risk for carriers [31]. A study by Ban et al. revealed an association between FokI polymorphism and glutamic acid decarboxylase autoantibodies (GAD65) positivity in a Japanese population [32]. However, the Type I Diabetes Genetics Consortium did not find any association of *VDR* SNPs with T1DM in the overall sample set, or in any of the subgroups analyses of the parent-of-origin, sex of offspring, and the human leukocyte antigen (HLA) risk [33]. Nevertheless, the FokI polymorphism of the *VDR* could have functional implications, altering ligand-mediated gene expression in beta-cells or the immune system [34]. Similar confusing data exist for linkage between T1DM and polymorphisms in the genes encoding enzymes with central roles in vitamin D metabolism, like *CYP27B1* or vitamin D binding protein (DBP).

In childhood diabetes, several epidemiologic studies describe a north-south gradient in the incidence of T1DM as well as a seasonal pattern of disease onset [35]. Dietary vitamin D supplementation is often recommended for pregnant women and children to prevent vitamin D deficiency, but studies on correlations between levels of vitamin D or even vitamin D supplementation in early life are confusing, with some showing a protective correlation with vitamin D supplementation or higher serum levels [36, 37], but others not [38]. A meta-analysis of four case-control studies and one cohort-study revealed that the risk of T1DM in later life was significantly reduced (29% reduction) in infants who were supplemented with vitamin D compared with unsupplemented controls [39].

Preclinical data point to a role for the vitamin D system in the pathogenesis of T1DM, as receptors for $1,25(OH)_2D_3$ are found both in beta-cells and most cells of the immune system. Wolden-Kirk et al. showed that $1,25(OH)_2D_3$ could almost completely prevent cell death induced by IL-1β and IFN-γ in human and mouse whole islets, while it restored impaired insulin secretion. Moreover, this protection was accompanied by alterations in gene expression of genes involved in chemotaxis, cell death and beta-cell function [40]. When studying islets from a vitamin D-sufficient donor, however, no improvement of insulin secretion is observed by incubating these islets with higher doses of vitamin D *in vitro* [41]. As previously said, effects of vitamin D on the immune system *in vitro* and in animal models of T1DM indicate that $1,25(OH)_2D_3$ is a potent immune modulator, with shifting of the cytokine profile of T cells towards a Th2 profile, induction of tolerance-inducing DC and induction of regulator T cells, both via direct effect on T cells and indirect effects on DC [39]. When high doses of vitamin D, $1,25(OH)_2D_3$ or vitamin D analogs are administered in an animal model of type 1 diabetes, the NOD mouse, these immune alterations can be picked up *in vivo* and diabetes can be prevented or its progression arrested [39].

Until now, clinical intervention studies using vitamin D or $1,25(OH)_2D_3$ (-analogs) in the prevention of T1DM or in people already affected with the disease have been disappointing. A small intervention trial in which new-onset diabetic children were given a small dose of $1,25(OH)_2D_3$ (0.25 μg/2d) or nicotinamide (25 mg/kg/d) showed that they had no improvements of C-peptide levels, although insulin requirements decreased in the $1,25(OH)_2D_3$-treated group [42]. Even when the dose was increased to 0.25 μg $1,25(OH)_2D_3$ daily for 2 years, given to recent-onset diabetic patients with high basal C-peptide levels, no protective effect was observed on HbA1c and insulin requirement. In a small prospective trial, 12 high-risk children with type 1 diabetes autoantibodies were treated with oral calcitriol (0.25 μg/d) for 1–3 years. Here, $1,25(OH)_2D_3$ was able to decrease serum autoantibody levels against GAD65 and insulin in all participants, suggesting some immune modulating effect [43]. The study was, however, too small to allow clinical conclusions. Patients with latent autoimmune diabetes in adults (LADA) who received $1\alpha(OH)D_3$, the synthetic precursor of $1,25(OH)_2D_3$, exhibited a partial preservation of beta-cell function in comparison to patients treated with insulin alone [44]. An open study in recent-onset diabetic patients with $1,25(OH)_2D_3$ (0.25 μg daily for 9 months, the maximum tolerable dose) revealed no significant safety issues as a result of the therapy but the treatment failed to induce preservation of beta-cell function [45]. No differences in area under the curve (AUC) of C-peptide, peak C-peptide, and fasting C-peptide levels between the treatment and placebo groups were observed at 9 and 18 months after study entry. Moreover, HbA_{1c} and daily insulin requirement were comparable between control and $1,25(OH)_2D_3$-treated patients throughout the study follow-up period.

At present, the advice to individuals at high genetic risk for developing T1DM should be to avoid vitamin D deficiency with adequate vitamin D supplementation, but at present data to advise higher supplements of vitamin D or interventions with high doses of $1,25(OH)_2D_3$ are lacking. Thus, clinical

studies indicating that the beneficial effects observed in animal models can be safely reproduced in humans are needed.

5 Vitamin D and Addison's disease

Addison's disease (AD) is a rare disorder characterized by autoimmune-mediated selective destruction of the adrenal cortex, which can be isolated (40% of patients) or associated with autoimmune polyendocrine syndromes (APS) (60% of patients) type 1, 2, or 4 [46]. The etiology of AD still remains largely elusive. Several genes, of which the HLA haplotypes are the most strongly associated, interact with environmental factors to confer disease susceptibility [47]. However, polymorphisms of *VDR* as well other genes involved in vitamin D metabolism are associated with AD [48–51]. A study by Pani et al. on distribution of four *VDR* polymorphisms (FokI, BsmI, ApaI, and TaqI) in 95 patients with AD in comparison to 220 healthy controls, demonstrated that the "ff" genotype of FokI and the "tt" genotype of TaqI were significantly more frequent in patients than in control group (13.7% versus 5.5%, OR) = 2.75 for "ff" genotype and 28.4% versus 14.1%, OR = 2.42 for "tt" genotype) [48]. They did not observe any difference in the other studied polymorphisms between patients and controls. The authors concluded that the "ff" and the "tt" genotype of the *VDR* gene may be associated with susceptibility of AD [48]. Another component of the vitamin D metabolism, the cytochrome P450 27B1 (*CYP27B1*), is associated with AD. The *CYP27B1* encodes the 1α-hydroxylase, the mitochondrial enzyme that catalyzes the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$. In particular, three different studies demonstrated a significant association between the *CYP27B1* promoter C(-1260)A polymorphisms and AD in German (OR 1.53, 95% CI 1.07–2.20) [49], British (OR 1.71, 95% CI 1.20–2.44) [51], and Polish population (OR 1.18, 95% CI 0.86–1.62) [50]. In the more recent of these studies, Fichna et al. performed also a meta-analysis of these three European cohorts, including a total of 325 patients affected by AD and 925 healthy controls. The meta-analysis showed in AD patients an overall OR of 1.44 (95% CI: 1.18–1.75) for the C(-1260)A allele [50]. Therefore, it was demonstrated that patients with APS associated with AD presented an increased frequency of the C(-1260) allele [52]. These results highlighted a potential role of *CYP27B1* polymorphisms with a favorable genetic background for various autoimmune disorders. Little is known about the role of this C(-1260)A allele; however, it seems that it may affect the *CYP27B1* transcription that causes a decrease of the availability of the active form of vitamin D [51].

Only few observational studies investigated the link between vitamin D plasma levels and AD. A record-linkage study by Ramagopalan et al. showed in a large cohort of patients admitted to a UK hospital for vitamin D deficiency, significantly elevated rates of AD (rate ratio = 7.0, 95% CI: 3.6–12.3) and of other autoimmune diseases [53]. Accordingly, Bellastella et al. demonstrated that patients with APS presented lower levels of 25(OH)D in comparison to healthy controls ($P < 0.001$) [54]. Moreover, they showed that the vitamin D status was not different in patients with single or multiple autoimmune diseases, but it changed depending on the type of autoimmune disease. The authors concluded that the presence of other autoimmune diseases, which could impair the absorption or the metabolic steps of vitamin D in the skin, liver, or kidney, may influence vitamin D levels more than AD [54].

It is important to note that in AD, the glucocorticoid deficiency may lead to suppression of the PTH–vitamin D axis [55]. In a small randomized trial involving nine patients with primary adrenal insufficiency, of whom eight were affected by AD, the authors showed that those patients who underwent all different schedules of glucocorticoid replacement therapy did not present suppressed levels of vitamin D [56].

At present, evidence regarding the association of vitamin D and AD is largely based on few observational studies. These preliminary results suggest that vitamin D may influence the genetic susceptibility of AD by modifying the immune response. However, further intervention studies are necessary to confirm or refute the correlation between vitamin D levels and AD.

6 Vitamin D and Hashimoto's thyroiditis

Several studies in the last few years have led to presume a link between vitamin D deficiency and autoimmune thyroid diseases, including Hashimoto's thyroiditis (HT) and Graves' disease (GD) [57]. A recent meta-analysis investigated the association between vitamin D level and autoimmune thyroid disease (AITD) through an accurate systematic literature review [58] concluded that serum 25(OH)D was lower in AITD patients compared with healthy control individuals (OR = 2.99, 95% CI: 1.88–4.74) and AITD was more likely to develop in individuals who showed low levels of serum 25(OH)D, thus suggesting that vitamin D deficiency may play a role in the pathologic process of AITD. This result was confirmed by several recent studies, which showed that vitamin D deficiency is more common in AITD patients, whether children [59] or elderly [60], both at low [61] and high latitude [62]. Moreover, a randomized controlled trial has recently found that vitamin D supplementation in AITD patients was correlated with significant reduction in anti-thyroperoxidase antibody (TPO-Ab) titers, leading to the idea that giving vitamin D to AITD patient can induce an improvement of the disease [63].

Other studies failed to establish a firm correlation. Effraimidis et al. [64] showed how vitamin D deficiency is

not associated with early stages of thyroid autoimmunity, while an Asian Indian community-based survey found only a weak inverse correlation between serum 25(OH)D values and TPO-Ab titers [65]. A further study showed, by measuring 25(OH)D level in a HT group and in a control group, that the mean 25(OH)D level for the female HT group (30.8 ± 7.5 ng/mL) was significantly higher than in the female control group (27.6 ± 8.1). In contrast with many other studies, it was observed that female HT subjects had both a higher rate of vitamin D sufficiency (51.7% versus 31.1%) and a lower rate of insufficiency (48.3% versus 68.9%). Of note, however, is that this difference may have been due to potentially increased vitamin D supplementation in HT females [66].

Growing evidence shows that the *VDR* polymorphisms are associated with an increased incidence of AITD. One study investigated the distribution of *VDR* alleles in a group of 111 Turkish patients with HT and 159 healthy controls. It showed that *VDR* gene TaqI TT and FokI FF genotypes were associated with increased risk of HT; BbAaTtFf genotype seemed to be protective for HT disease in the same population [67].

In another study, it was tested if the functional *VDR* polymorphisms (TaqI rs731236, ApaI rs7975232, FokI rs2228570, and BsmI rs1544410) are involved in the pathogenesis of AITD [68]. Using polymerase chain reaction-restriction fragment length polymorphism, 139 Graves' disease patients, 116 HT patients, and 76 control subjects were genotyped. The frequency of the TT genotype for the TaqI polymorphism was higher in GD patients than in HT patients ($P = 0.0147$). The frequency of the C allele for the ApaI polymorphism was higher in AITD patients than in control subjects ($P = 0.0349$). Focusing on HT, the frequency of the CC genotype for the FokI polymorphism was higher in HT patients than in control subjects ($P = 0.0174$). Because the C allele was also associated with a higher production of IL-12, which induces Th1 differentiation and thyroid destruction in HD patients [34], the CC genotype may be associated with the induction of autoimmune thyroid destruction. No differences were found in the frequencies of the genotypes and alleles of the BsmI polymorphism between the control subjects and the HT patients. Except for BsmI polymorphism, this experiment showed how genetic differences in the *VDR* gene may be involved in the development of AITD. Conflicting evidence was observed in a meta-analysis of eight studies, in which the result indicates that both BsmI and TaqI polymorphisms are significantly associated with AITD risk (Pz = 0.001 for B versus b; Pz = 0.010 for t versus T), but not the ApaI or FokI polymorphisms. In the subgroup analysis in Europeans, the decreased risk of AITD remained for the B or t variant [69]. This gene-based analysis indicates that based on current evidence from published studies, the cumulative effect of BsmI or TaqI polymorphisms in *VDR* is significantly associated with AITD [69]. This result was not confirmed by Giovinazzo et al. [70], who observed that the genotype distribution of the *VDR* single nucleotide polymorphisms (SNPs) was not different between HT patients and healthy individuals (BsmI $P = 0.783$; ApaI $P = 0.512$; TaqI $P = 0.471$). However, even if the *VDR* locus does not appear to be involved in conditioning the genetic susceptibility of HT, vitamin D deficiency may likely contribute to the disease development and progression, acting as an environmental trigger.

Thus, controversial opinions on vitamin D role in AITD onset are expressed by the scientific community. Several cofactors may affect the results of epidemiologic studies, such as sun exposure, obesity, sedentary life, leading to contradicting results. However, the growing evidence of a correlation between low levels of vitamin D and AITD suggests the advisability of supplementation.

7 Vitamin D and Graves' disease

Current evidence suggested that vitamin D deficiency might cause the onset and/or development of different organ-specific and systemic autoimmune diseases [71]. GD is one of the most frequent diseases among autoimmune disorders, with an annual incidence of approximately 14 per 100,000 [72]. GD is an autoimmune thyroid disease in which thyroid-stimulating hormone (TSH) receptor autoantibodies cause hyperthyroidism [73]. Besides thyrotoxicosis, the clinical manifestations of GD include several extra-thyroidal signs, such as ophthalmopathy, dermopathy, and acropathy [74]. Experimental and clinical evidence supports that GD results from complex interactions between genetic and environmental factors that lead to the loss of immune tolerance to thyroid antigens and the initiation of an immune reaction [75, 76]. GD occurs with the infiltration of T cells in the thyroid gland. In particular, TH cells elaborate various cytokines, including IFN-γ, which induce in thyrocytes the expression of HLA-DR antigens. The expression of HLA-DR antigens on thyroid follicular cells triggers an autoimmune process and renders them susceptible to immunologic attack [77].

Although vitamin D is commonly included among the environmental factors responsible for the immunopathogenesis of AITD, the association between vitamin D status and GD is not so straight forward [78]. Two recent meta-analyses addressed the association between vitamin D and GD. Besides the above-mentioned systematic review of Wang et al. [58], Xu et al. [79] in a meta-analysis including 26 studies showed that low vitamin D status may increase the risk of GD. In particular, patients with GD were more likely to be deficient in vitamin D compared with the controls (OR = 2.24, 95% CI: 1.31–3.81) [79]. Nevertheless, it should be noted that this association, even supported by a stronger statistical significance compared with previous studies, does not necessarily imply a causal relationship between vitamin D status and GD [80].

Choi et al. [81] reported that serum 25(OH)D_3 levels were significantly lower in premenopausal women with TPO-Ab than in women without TPO-Ab, with a prevalence of 21.2%, 15.5%, and 12.6% in women with vitamin D deficiency, insufficiency, and sufficiency, respectively. However, a prospective study performed within the Amsterdam AITD cohort, in which controls were matched to cases for age, body mass index, smoking, estrogen use, season, and duration of follow-up did not confirm these data, in that 25(OH)D_3 and serum 1,25$(OH)_2D_3$ concentrations were not different between cases (defined as those subjects in whom TPO-Ab developed de novo during follow-up) and controls, neither at baseline nor at the time of the occurrence of TPO-Ab [64].

The issue of the relationships between vitamin D and GD may become even more complicated by the finding that, as above mentioned, particular polymorphisms in the *VDR* gene are associated with AITD [69]. Human immune cells, including macrophages, dendritic cells, T and B lymphocytes, are known to express the vitamin D-activating enzyme CYP27B1 and the VDR, an intracellular receptor belonging to the steroid/thyroid nuclear receptor family [58, 82]. Thus, altered activities of polymorphic variants of *VDR* may affect immune cells interaction with vitamin D. Similarly to what was found in the Hashimoto's thyroiditis, specific *VDR* gene polymorphisms were found to be associated with susceptibility to GD in a number of different investigations, but the statistical power of most studies was very low. A comprehensive meta-analysis by Zhou et al. analyzed the associations among four polymorphisms of the *VDR* gene (ApaI, TaqI, BsmI, and FokI) and susceptibility to GD in a total of 1820 GD patients and 2066 controls from Caucasian and Asian populations [83]. ApaI, BsmI, and FokI polymorphisms in the *VDR* gene resulted associated with susceptibility to GD in Asian populations, whereas ApaI, BsmI, TaqI, and FokI polymorphisms were not associated with GD in Caucasian populations. Thus, associations or linkage of *VDR* gene polymorphisms with GD found in same reports have been difficult to replicate in other populations and the mechanisms by which these variants associate with the disease susceptibility remain largely elusive.

In conclusion, an association between low vitamin D levels and thyroid autoimmunity is likely. However, whether vitamin D deficiency plays a causative role in the onset of the disease needs to be unravelled. In addition, so far there have been no definitive studies to evaluate the effect of vitamin D supplementation on thyroid autoimmunity. To confirm any causality link between vitamin D and GD, more cohort or intervention studies are needed to evaluate whether vitamin D supplementation decreases the risk of thyroid autoimmunity [78].

8 Vitamin D and autoimmune polyendocrine syndromes

The APS include different conditions characterized by the coexistence of at least two endocrine or non-endocrine autoimmune-mediated diseases [84].

According to the accepted criteria of classification, APS are distinguished in four main types. APS-1, characterized by the presence of chronic candidiasis, chronic hypoparathyroidism and AD, is a very rare syndrome that affects young subjects and is caused by different mutations of the autoimmune regulator (*AIRE*) gene localized on chromosome 21 [85]. APS-2, characterized by the presence of AD (always present), autoimmune thyroid diseases, and/or T1DM, is also a rare syndrome affecting particularly adult females and is associated to a genetic pattern of HLA DR3/DR4. Autoimmune thyroid diseases associated to other autoimmune diseases (excluding AD and/or hypoparathyroidism) are the main characteristics of APS-3. The different clinical combinations of autoimmune diseases not included in the previous groups are characteristics of APS-4 [84].

However, only one study has evaluated whether the association of APS affects the vitamin D status [54]. As already discussed above, this study reported that a higher prevalence of low vitamin D status was observed in those with APS-3. In addition, lower vitamin D concentrations were found among patients either with a single autoimmune disease, such as T1DM, or with APS including T1DM, compared with control subjects. This finding suggested that the kind of autoimmune disease rather than the association of several autoimmune diseases, as happens in APS, may influence negatively vitamin D status of affected patients, likely linked to an impairment of the absorption or the metabolic steps of this vitamin at the skin, liver, or kidney level [54].

In conclusion, further prospective studies are needed to clarify if impaired vitamin D status is a causal factor in the pathogenesis of APS or a consequence of them.

9 The role of vitamin D supplementation in the prevention of autoimmune diseases

Blood concentration of 25(OH)D is the biomarker usually used by clinicians and researchers to determine vitamin D status [4]. At this time there is no international consensus on the optimal concentration of vitamin D to prevent deleterious consequences in non-classic vitamin D pathways. The Institute of Medicine (IOM) guideline and the National Osteoporosis Society guideline consider 20 ng/mL (50 nmol/L) a sufficient concentration of total vitamin D (25OHD) to achieve appropriate bone health. Vitamin D deficiency is defined as 25OHD level less than 12 ng/mL (30 nmol/L) [86, 87]. The Endocrine Society derived different

thresholds, recognizing sufficient 25-OH vitamin D levels greater than 30 ng/mL (75 nmol/L), insufficient levels ranging from 20 to 29.9 ng/mL (52–72 nmol/L), and deficient vitamin D levels less than 20 ng/mL (50 nmol/L) [88]. The differences between these guidelines are explained by the investigated target population; particularly, the IOM guidelines are based largely on vitamin D effects on bone and mineral homeostasis in the general healthy population, whereas the Endocrine Society guidelines are based on observational and clinical trials on populations with high risk for vitamin D deficiency. Therefore, the optimal 25(OH)D levels to prevent the onset of autoimmune diseases are still under debate [3, 86, 88]. However, authors suggest vitamin D levels higher than 30 ng/mL might be needed in order to reach positive effects [88, 89].

Similar to what has been reported for optimal vitamin D levels, there also is no consensus for the optimal amount of vitamin D supplementation. *In vivo* studies on animal models showed that the administration of vitamin D3 arrested immunologic progression and prevented the clinical onset of autoimmune diseases such as T1DM [90]. Moreover, experimental studies in humans showed beneficial effects of vitamin D supplementation in reducing the risk of developing autoimmune disease [39] and in reducing the severity of disease activity [91].

Nevertheless, oral vitamin D intake in many of the studied populations could be too low to produce significant effects; further, variability in administration may reduce positive effects. In the evaluated observational studies, the difference between the higher and the lower oral doses of vitamin D administration is mostly 400 IU/d (10 μg/d). It is important to note that the achievement of optimal 25(OH)D levels depends on both the baseline serum level and the chosen target level [92]. Heaney et al. reported a linear correlation between serum 25(OH)D levels and vitamin D dosing with a coefficient of determination of 0.7 nmol/L [92]. For example, to reach and maintain a sufficient serum 25(OH)D level of 80 nmol/L from a vitamin D deficiency baseline of 60 nmol/L, one would need an additional intake of 29 μg (1160 IU) daily of vitamin D, whereas from a deficiency baseline 25(OH)D level of 40 nmol/L, one would need a supplementation of about 55 μg (2200 IU) daily of vitamin D [93]. However, there is a steeper rise in serum 25(OH)D levels when vitamin D administration dosing is less than 1000 IU/d; a slower, more flattened response is seen when doses of 1000 IU/d or higher are administered. Thus, when the supplementation dose of vitamin D is ≥1000 IU/d, the rise in serum 25(OH)D is approximately 1 nmol/L (0.4 ng/mL) for each 40 IU of intake, whereas when the dose is ≤600 IU/d, the rise is serum 25(OH)D is approximately 2.3 nmol/L for each 40 IU [92]. Thus, when the ingested dose of vitamin D is more than 1000 IU per day, a difference between the various dosages reported from the studies of 10 μg (400 IU) per day corresponds to a difference in 25(OH)D levels of only 10 nmol/L (4 ng/mL) [94, 95]. This increase in serum 25(OH)D levels of 10 nmol/L observed among different studies might be too low to achieve the expected outcome. Indeed, it is necessary to reach 25(OH)D levels greater than 75 nmol/L to obtain health benefits [88]. Thus, it is often required to ingest vitamin D dosages of at least 20–25 μg (800–1000 IU) per day for patients with insufficient 25(OH)D levels at baseline [94, 96–98]. The Endocrine Society guidelines suggest a high intake of 50,000 IU of vitamin D once a week for 8 weeks (6000 IU) per day to reach sufficient 25(OH)D blood levels, followed by maintenance administration of 1500–2000 IU (37.5–50 μg) per day in all adults who are vitamin D deficient [88, 99].

Most studies suggest an acute vitamin D intoxication for serum 25(OH)D levels >150 ng/mL, characterized by hypercalcemia, hypercalciuria and calcifications in different organs [3, 100–102]. However, the great majority of vitamin D intoxication cases are due to a prolonged intake of >40,000 IU/d [103]. The IOM suggests a supplementation of vitamin D of maximum 4000 IU/d [86], whereas the Endocrine Society recommends a maximum supplementation of 10,000 IU/d. [88].

Because of the potential side effect of activated vitamin D, cholecalciferol is the preferred form for supplementation. In comparison to the other inactive forms of vitamin D (vitamin D_2 or ergocalciferol), cholecalciferol has a longer plasma half-life [104] and a higher tissue bioavailability [105].

It appears necessary to evaluate through controlled randomized studies both the best kind of vitamin D compound and the appropriate dose to prevent insufficient vitamin D levels, in order to control the autoimmune mechanisms [91].

10 Conclusion

In the last few years, more attention has been given to the "non-calcemic" effects of vitamin D. Several observational studies and meta-analyses demonstrated an association between circulating levels of vitamin D with autoimmune endocrine disorders, including T1DM, AD, AITD, including HT and GD, and autoimmune polyendocrine syndromes [5]. These findings are supported by the expression of the 1α-hydroxylase and the VDR in cells of the immune system [9]. The expression of the enzyme involved in the activation of the vitamin D suggests a local action of vitamin D, which presents immunomodulatory properties. Thus, it is possible that vitamin D insufficiency or deficiency may unsettle the normal immune response, predisposing to the development of the autoimmune diseases. Moreover, the association between both *VDR* and *CYP27B1* polymorphisms and a higher risk of T1DM, AD, and AITD [34, 48, 50, 68] strengthens the potential role of vitamin D in the pathogenesis of autoimmune endocrine diseases.

Currently, the optimal plasma 25(OH)D concentration that is necessary to prevent or treat autoimmune diseases is still under debate. However, experimental studies in humans have indicated the beneficial effects of vitamin D supplementation in reducing the severity of disease activity [91]. Similarly, studies on animal models showed that the administration of vitamin D could prevent the development of autoimmune diseases [91].

It is important to note that the described association between low levels of vitamin D and autoimmune endocrine diseases is mostly derived from *in vivo* animal models or observational studies, which could present several bias. Therefore, the observed low levels of vitamin D could be caused by inflammatory state and/or less time spent outdoors by the individuals because of their underlying disease. Further randomized controlled trials with a long period of follow-up are necessary to establish causality between vitamin D and autoimmune endocrine diseases and to provide information about the potential role of vitamin D supplementation in the prevention of these autoimmune diseases.

Authors contribution B.A. and G.M. designed the study. B.A., G.M., L.B., C.M., C.V.V., L.M. and G.B. participated in the literature search and performed the selection of studies. B.A. wrote the Introduction, Methods and the Conclusion of the review, as well as the paragraphs on Addison's disease and on vitamin D supplementation. C.V.V. supported B.A. in writing the Introduction and the paragraphs on vitamin D supplementation. G.M., S.S., N.B. and C.L.R. collaborated to the preparation of the manuscript providing relevant suggestions and corrections according to their long-lasting expertise in different research fields. L.B. wrote the paragraph on Grave's disease and autoimmune polyendocrine syndromes. C.M. wrote the paragraph on diabetes mellitus. L.M. prepared the figure and wrote the paragraph on clinical and basic evidence. G.B. wrote the paragraph on Hashimoto thyroiditis. V.M.A., G.T. and G.B. contributed to the final version of the manuscript. A.C., A.P. and S.D.C. coordinated and supervised the preparation of the manuscript. All the authors reviewed and approved the final version of the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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