Review

Vitamin D and diabetes mellitus

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ABSTRACT

The vitamin D endocrine system in now recognized as subserving a wide range of fundamental biological functions in cell differentiation, inhibition of cell growth as well as immunomodulation. Both forms of immunity, namely adaptive and innate, are regulated by $1,25(OH)_2D_3$. The immune-modulatory properties of vitamin D suggest that it could play a potential therapeutic role in prevention of type 1 diabetes mellitus (T1DM). It is postulated that large doses of vitamin D supplementation may influence the pattern of immune regulation and subsequent progression to T1DM in a genetically susceptible individual. More studies are required to substantiate the relation between T1DM and vitamin D/vitamin D analogues in the pattern of immune regulations in susceptible individuals. In type 2 diabetes mellitus (T2DM), vitamin D may influence both insulin secretion and sensitivity. An inverse relationship between T2DM and vitamin D is postulated from cross-sectional and prospective studies, though conclusive proof is as yet lacking. Available studies differ in their design and in the recommended daily allowances (RDA) of vitamin D in non-skeletal diseases and β-cell function. Large, well designed, controlled, randomized interventional studies on the potential role of vitamin D and calcium in prevention and management of T2DM are required to clarify the relationship between vitamin D and glucose homeostasis in T2DM.

Key words: β-cell function, Immune functions, Insulin secretion, Insulin sensitivity, Type 1 diabetes mellitus, Type 2 diabetes mellitus, Vitamin D

INTRODUCTION

Vitamin D has long been known as the anti-rickets factor or sunshine vitamin. Today, vitamin D defi-

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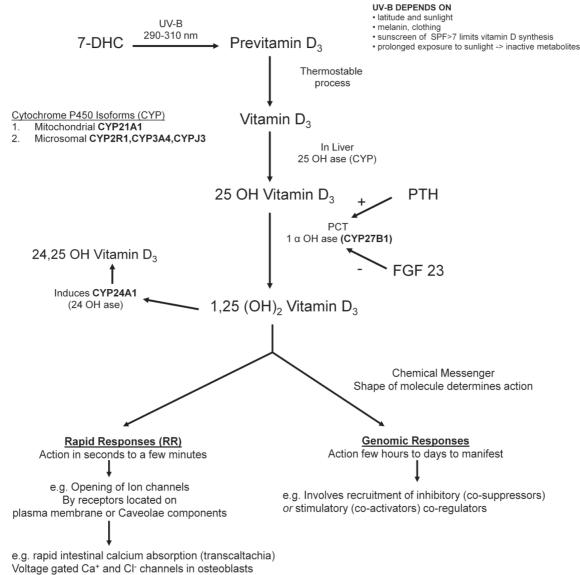E-mail: cvhari5endo@rediffmail.com

ciency is considered to be a global health problem. In 2008, it was estimated that one billion individuals have vitamin D deficiency (25OH Vitamin D <20 ng/ml).[1] Meanwhile, type 2 diabetes (T2DM) is one of the main non-communicable diseases. It is estimated that 366 million people had diabetes in 2011 and that by 2030 this may rise well above half a billion cases.[2]

Vitamin D synthesis

Vitamin D_2 is ergocalciferol derived from plant and vegetable sources. Vitamin D_3 is a secosteroid synthesized in the skin from 7-dehydrocholetserol (7-DHC) on exposure to sunlight by ultraviolet B-rays (UV-B

rays 290-310 nm) (Figure 1),[3,4] while melanin, skin pigmentation, clothing and sunscreen limit vitamin D_3 production. Intensity of UV-B radiation of sunlight varies according to latitude. Hence, the farther one lives from the equator, the less UV-B is available from sunlight for induction of vitamin D synthesis

Figure 1. Vitamin D_3 is secosteroid synthesized in the skin from 7-dehydrocholetserol (7-DHC) through a two step process. The B-ring of the 7-DHC is broken under UV-B rays (290-310 nm) forming pre-vitamin D_3 that isomerizes to vitamin D_3 in thermo-sensitive but non-enzymatic process. Vitamin D_3 needs to be hydroxylated twice to become biologically active. The enzyme 25 OHase in the liver converts vitamin D_3 into 25OHD.[11] Serum 25OHD$_3$ is a reliable indicator of vitamin D status.[12] Vitamin D is bound to a carrier protein, the vitamin D binding protein (DBP), and transported through the circulation. In proximal tubules (PCT) of the kidney, second hydroxylation occurs by 1-α-hydroxylase (1αOHase, CYP27B1) into 1, 25 dihydroxy vitamin D_3 [1,25(OH)$_2$D$_3$] which is the active form of vitamin D. 1,25(OH)$_2$D$_3$ generates biological response by the presence of its cognate receptors in selected target organs and tissues. The genomic responses of 1,25(OH)$_2$D$_3$ are mediated by the formation of their ligand-receptor complex with their cognate nuclear receptor. 1,25(OH)$_2$D$_3$ serves as a chemical messenger that transmits signals and rapid responses (RR) (e.g. opening of ion channels). A number of extra-renal sites such as immune cells epithelia of skin, gut, prostate, lung, bone, parathyroid gland and pancreatic islets may provide 1,25(OH)$_2$D$_3$ for local use as an intracrine or paracrine factor.[13,14] Control of 1,25(OH)$_2$D$_3$ by non-renal tissues depends on the organ concerned. In macrophages and keratinocytes, CYP27B1 is induced by invading organisms.[15]

in the skin.[5] Vitamin D needs to be hydroxylated twice to become biologically active: it must first be converted to 25 hydroxyvitamin D_3 ($25OHD_3$) and further to $1,25(OH)_2$ vitamin D_3 to be functionally active (Figure 1).

Tissue-specific actions of vitamin D

The genomic responses generally take a few hours to days to become fully manifest, the binding of $1,25(OH)_2D_3$ ligand to VDR triggering a tight association with its heterodimeric partner retinoid X receptor (RXR). Only the $1,25(OH)_2D_3$-RXR-VDR complex is able to penetrate the deep groove of DNA and recognize vitamin D responsive elements (VDREs) located within introns and/or at large distances from the transcription start site.[6] The control of transcription requires additional recruitment of co-regulators that can be inhibitory (co-suppressors) or stimulatory (co-activators).[7,8] While certain genes are selective for the co-regulator that combines with VDR and regulates their transcription, these genomic responses can be blocked by inhibitors of transcription and translation.[8]

$1,25(OH)_2D_3$ serves as a chemical messenger that transmits signals and rapid responses (RR) (e.g. opening of ion channels), the RR being mediated by a variety of receptors located near or associated with plasma membrane or its caveolae components.[9] Caveolae are flask-shaped membrane invaginations that are enriched in spingolipids and cholesterol commonly found in both caveolae and/or lipid rafts.[10] The time required for RR varies from seconds (opening of ion channels) to 10-60 minutes (e.g. activation of phosphotidylinsoitol-3'-kinases, endothelial nitric oxide synthatase). Examples of RR include rapid intestinal absorption of calcium (transcaltachia), secretion of insulin by pancreatic β-cells, opening of voltage-gated Ca^+ and Cl^- channels of osteoblasts and rapid migration of endothelial cells.[9,10] Interestingly, one isomeric form of $1,25(OH)_2D_3$ is used for genomic response and a different isomeric form serves as an agonist of rapid response.[10] The ability of individual tissues to produce their own $1,25(OH)_2D_3$ in a tissue-specific fashion may account for the great specificity with which vitamin D regulates numerous functions in many tissues (Figure 1).

VITAMIN D AND REGULATION OF IMMUNE FUNCTION

It is now widely recognized that the vitamin D endocrine system subserves a wide range of fundamental biological functions in cell differentiation, inhibition of cell growth as well as immunomodulation. Both forms of immunity, namely adaptive and innate, are regulated by $1,25(OH)_2D_3$ (Figure 2). VDR are found in activated dendritic cells, macrophages and lymphocytes. These cells produce $1,25(OH)_2D_3$ (expressing CYP27B1 which converts 25(OH)D to $1,25(OH)_2D_3$).[16] $1,25(OH)_2D_3$ regulates their proliferation and function.[17,18]
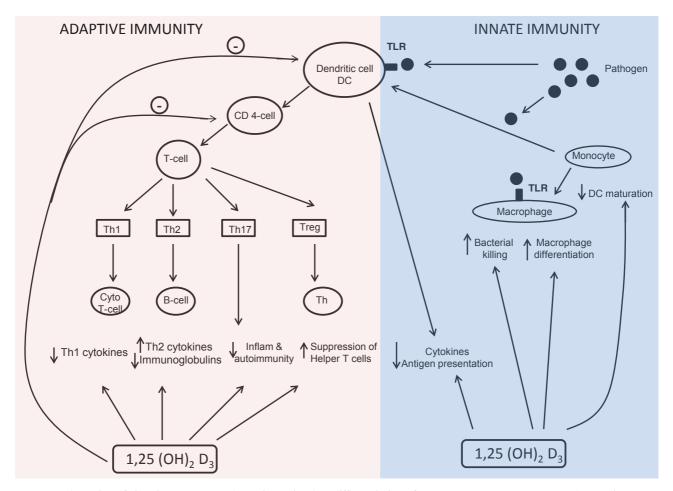
Vitamin D and innate immunity

Cytotoxic T lymphocytes (cytotoxic T-cells), Natural Killer cells (NK cells), macrophages and monocyte precursors play a central role in initial nonspecific response to tissue damage or pathogenic organisms, i.e. cell-mediated immunity. They do this by phagocytosis of cellular debris or pathogens and by assimilating the resulting waste material or eliminating it. The macrophages use the phagocytic material for antigen presentation to T-cells (Figure 2), this function involving activating toll-like receptors (TLRs) in polymorphonuclear cells (PMN).

Experimental studies have confirmed that 25(OH)D_3 is able to induce intracrine VDR responses in monocytes which have been treated with TLR2/1 activator. The TLR2/$1,25(OH)_2D_3$ combination stimulates the expression of antibacterial protein cathelicidin.[19] The beneficial role of vitamin D in innate immune regulations consists in its contribution to the regulation of feedback control pathways. $1,25(OH)_2D_3$ is shown to down-regulate the expression of the monocytes TLR2 and TLR4, thereby suppressing inflammatory response by using these receptors.[20] Thus, by activating CYP24 and TLR regulating mechanisms, vitamin D prevents over-elaboration of innate immune response and the damage associated with it.

Vitamin D and dendritic cells - Antigen presentation

Antigen presenting cells (APC), and specifically dendritic cells (DCs), present antigens to cells involved in the adaptive arm of the immune system. DCs, which are derived from monocytes and are

Figure 2. The action of vitamin D on macrophages is to stimulate differentiation of precursor monocyte to mature phagocytic macrophages.[17,18] The dendritic cells stimulate the effector CD4+ cells to differentiate into one of the four types of T-helper cells (Th cells). Activated T-cells express VDR. $1,25(OH)_2D_3$ inhibits the development of T-helper 1 (Th1) cells which are associated with cellular, immune response.[28] $1,25(OH)_2D_3$ promotes T-helper 2 (Th2) cells associated with humoral (antibody) mediated immunity.[33] Thus, $1,25(OH)_2D_3$ promotes the T-cell shift from Th1 to Th2 cells cell function.[35] $1,25(OH)_{2D3}$ inhibits the development of T helper 17 (Th17) cells which plays an essential role in combating certain pathogens and also linked to tissue damage and inflammation.[35] The fourth group of CD4+ T-cells, the Treg cells exerts suppressor functions.[36] *(Adapted from Martin Hewison, 2010 Endocrinol Metab Clin North Am 39: 365-379).*

heterogeneous in terms of their phenotype, function and location, are classified into two groups based on their origin: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). mDCs are the most effective antigen presenting cells, while pDCs are associated with immune tolerance. They produce different types of cytokines and chemokines and exert complimentary effects on T-cell responses.[21] $1,25(OH)_2D_3$ regulates mDCs and suppresses activation of naove T-cells.[21]

Dendritic cells prime CD4+ T-cells (Figure 2). DCs capture and process antigen, and once this has been effected, the DCs will mature and increase expression of co-stimulatory molecules which are necessary for interaction between the T-cell receptor and the antigen/MHC complex.[22] Hampering the co-stimulatory capacity of DCs will lead to a shift from immunogenicity to tolerance. Since $1,25(OH)_2D_3$ suppresses the expression of MHC-II molecules as well as co-stimulatory molecules,[22,23] it can thus be deduced that $1,25(OH)_2D_3$ has immunosuppressive properties. It not only inhibits maturation of DCs but also increases apoptosis of mature DCs.[24] A study has shown that $1,25(OH)_2D_3$ and its synthetic analogues inhibited the maturation of monocyte-derived DCs,

thereby suppressing the capacity to present antigen to T-cells.[25] Extensive proteomic analysis of DCs with 14-epivitamin D_3 analogue (TX527) demonstrates that DCs are locked in an immature state and adopt a tolerogenic phenotype with a special endocytic property, as compared with mature or immature DCs.[24,26] Hence, $1,25(OH)_2D_3$ acts on dendritic cells to reduce their maturation and antigen presenting capability. Based on these data, it was proposed that vitamin D could act to promote immune tolerance, and this indeed was shown in studies of pancreatic islet cell transplantation where lower rejection rates were observed.[27] This in turn was associated with enhancement of suppressor or regulatory T-cells (Tregs), the latter accounting for the fact that treatment of monocyte-derived DCs in culture with $25OHD_3$ suppresses DCs maturation and inhibits T-cell proliferation.[27]

Vitamin D and adaptive immunity

The adaptive immune response involves the ability of T-cells to produce cytokines and of B lymphocytes to produce immunoglobulins specifically combating the source of antigen presented to them by either macrophages or dendritic cells. When activated, $1,25(OH)_2D_3$, which, exerts its inhibitory action on adaptive immunity,[28] induces proliferation and differentiation of T-cells (CD4+ and CD8+) along different pathways. $1,25(OH)_2D_3$ can induce preferential differentiation of Treg cells, a pivotal mechanism linking vitamin D and adaptive immunity[29] (Figure 2). $1,25(OH)_2D_3$ increases interlukin-10(IL-10) secretion and TLR9 expression via Treg, suggesting a link between innate and adaptive immune response.[30] The effect of vitamin D on CD8+ suppressor T-cells is limited. However, $1,25(OH)_2D_3$ actively regulates cytokine production by CD8+ cells and regulates proliferation of CD8+ cells following specific antigen stimuli.[31]

$1,25(OH)_2D_3$ inhibits cytokine secretion associated with Th1(IFN-γ) cells.[28] It also inhibits IL-12a T-cell stimulatory factor involved in differentiation of naïve T-cells into Th0 cells which further develop into Th1 cell or Th2 cells.[32] Vitamin D down-regulates pro-inflammatory cytokines, such as IL-2, IL-6, IFN-γ and tumor necrosis factor (TNF)-β while enhancing anti-inflammatory cytokines like IL-4, IL-10 and TGF-β.[33]

Active B lymphocytes express VDR, while rest-

ing B lymphocytes do not. $1,25(OH)_2D_3$ decreases proliferation of B lymphocytes and immunoglobulin and induces apotosis.[35] Given that indirect mediation by T-cells and monocytes or macrophages are the most important mechanisms of action,[34,17] hormonal vitamin D retards differentiation of β-cell precursors into plasma cells and suppresses proliferation and immunoglobulin production. This is beneficial for conditions in which the immune system is directed against the body's own tissues, i.e. autoimmunity (Figure 2).

DIABETES MELLITUS AND VITAMIN D SUPPLEMENTATION

A PubMed search was conducted using the terms "Vitamin D-insulin resistance–type 1 and 2 diabetes mellitus", "Vitamin D insulin–sensitivity–type 1 and 2 diabetes mellitus", "Vitamin D insulin–secretion–type 1 and 2 diabetes mellitus" under the category of clinical trials from the year 1985 till now. These references are tabulated in the present review.

Vitamin D in type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) is a chronic disease of multifactorial nature resulting from progressive autoimmune destruction of pancreatic islet cells at the early stages of disease.[37] Genetic predisposition and environmental factors are both contributory to the development of the disease. It is hypothesized that vitamin D may have a therapeutic role in T1DM via its immune-modulatory properties. More specifically, at the time of the onset of T1DM, chronic inflammatory insulinitis (infiltrates) is found in the islets of Langerhans involving CD8+ and CD4+ T-cells, B lymphocytes and macrophages.[37] Vitamin D inhibits the production of IFN-γ and IL-2 cytokines, known to activate both macrophages and cytotoxic T-cells, which leads to destruction of pancreatic islet cells.[38] Furthermore, vitamin D suppresses APC and modulates development of CD4+ lymphocytes.[39] Exposure of pancreatic β-cells to proinflammatory cytokine induces endoplasmic reticulum stress and apotosis.[40] $1,25(OH)_2D_3$ protects β-cells by reducing exposure of MHC-1 molecules.[41] It induces antiapoptotic A20 protein and decreases exposure of a transmembrane cell surface receptor, thus transducing apoptotic death signals, thereby contributing to the pathogenesis of

T1DM, namely, Fas.[42,43] Ecologically, observational studies have been conducted that showed a north-south gradient and that have linked the incidence of T1DM with latitude and seasonal pattern,[44] suggesting an inverse correlation between effective exposure of sunlight and incidence of T1DM.

Animal experiments

As demonstrated in animal models of T1DM non-obese diabetic mice (NOD mice), high doses of $1,25(OH)_2D_3$(5 μg/kg/alternate day) suppresses insulitis and diabetes development, this accompanied by a diminished number of effector T-cells as well as induction of Treg cells, which was shown to be the basis of protection.[43,45] In an inflammation-driven model of diabetes (streptozotocin-induced diabetes model), $1,25(OH)_2D_3$ reduced the incidence of diabetes but had little effect in reverting overt diabetes.[46] Pharmacological doses of $1,25(OH)_2D_3$ lead to hypercalcemia and bone calcification.[46] Use of structural analogues of $1,25(OH)_2D_3$ could partly solve this issue. It was recently observed that a combination of 14-epivitamin D_3 analogue (TX527) with cyclosporine (IFN-β) induced delay in recurrence of diabetes after islet transplantation with increased expression of IL-10 in islet cell grafts.[46] An analogue of vitamin D (KH1060) has been shown to prevent the onset of diabetes in the NOD mouse. Overall, animal studies suggest a halt of the progression of T1DM by vitamin D analogues even after initiation of an autoimmune attack, since administration of MC 1288 with or without cyclosporine A in established insulitis resulted in reduction of diabetes incidence.[47]

Studies in humans

Small-scale epidemiological clinical interventions have produced interesting findings. High doses of vitamin D (2000 IU/day) during the first year of life reduced the risk of development of T1DM.[48] Studies by Stein et al[48] have demonstrated that use of cod liver oil from 7-12 months of life was associated with lowered risk of developing T1DM in later life.[48] Also, cod liver oil taken by pregnant mothers in the 3rd trimester of pregnancy was associated with decreased risk of T1DM.[48] The European Community-sponsored Concerted Action on the Epidemiology and Prevention of Diabetes (EURODIAB) revealed that there was a 33% risk reduction (OR 0.67) of T1DM in children who received vitamin D supplementation in earlier life compared to non-supplemented subjects.[49,50] A meta-analysis of four case-control studies and one cohort study showed a 29% risk reduction in infants who were supplemented with vitamin D as compared with those who were not [pooled odds ratio(OR) 0.71, 95% confidence intervals (CI) 0.60-0.84].[49,50] The screening for T1DM genetic risk in newborns of an Italian study (Prevefin Italy)[51] sought to validate the efficacy of two primary prevention strategies (vitamin D supplementation and β-casein-free diet) in preventing autoimmune aggression of pancreatic β-cells and ultimately the onset of T1DM. Cord blood samples of 9409 Caucasian newborns were screened for risk of T1DM and HLA class II markers. Seventy-three newborns qualified for the study. Informed consent was obtained for those with a high genetic risk of T1DM and, after β-cell autoantibody analysis (GAD antibodies, IAA, IA-2A), were randomized into two arms: one arm to receive 500 IU of vitamin D free diet and the second arm to receive 500 IU of vitamin D plus β-casein-free diet in the first 12 months of life. Every three months, apart from clinical and auxiological examination, antibody analysis was also carried out. If antibodies were positive, they were metabolically tested with the glucagon test. The preliminary study showed a low rate of transient or persistent antibody positivity. Long-term results are awaited.[51] Studies in patients with Latent Autoimmune Diabetes in Adults (LADA) developing T1DM during treatment with analogues of vitamin D in addition to insulin treatment exhibited a better ability to preserve β-cell function compared with those treated with insulin alone.[52] Studies in pregnant women have displayed these beneficial effects in the form of reduced occurrence of diabetes-specific autoantibodies in offspring of mothers supplemented with vitamin D.[53]

While the immune protective effect of vitamin D is thought to be mainly based on Treg cells, these therefore constituting a central player in maintenance of self-tolerance, there are an insufficient number of studies on the effect of vitamin D supplementation on peripheral T-cells. Recent studies of Bock et al[54] showed that supplementation with oral vitamin D at doses of 140,000 IU monthly for three months significantly increased the Tregs after this short period. These results indicate that vitamin D may be a useful therapeutic agent exerting immune modulatory ef-

fects that involve a stimulatory effect on Tregs. Table 1 presents randomized clinical trials that studied the effect of vitamin D on established T1DM and their outcomes.[55-59]

In summary, the proven immune modulatory effect of vitamin D strongly points to a rational hypothesis for its role in the pathogenesis of type 1 diabetes, one of the most common autoimmune diseases, this being

Table 1. Clinical trials on vitamin D intervention in Type 1 diabetes mellitus

S.No	Year	Country	Participants (Number of participants)	Age	Vitamin D form and dose intervention	Study duration	Out come	Reference
Positive out comes
1	2012	Brazil	New onset T1DM with fasting serum C-peptide >0.6 ng/ml (n=38)		Oral cholecalciferol 2000 IU/day	Mar 2006 to Oct 2010	Protective immunogenic effect and slow decline of residual β-cell function. Chemokine ligand -2 and regulatory T-cell percentage higher than placebo	Gabbay et al[55]
2	2010	Saudi Arabia	T1DM with 25 OHD levels <50 nmol/L (n=80)		4000 IU of Vitamin D_3 and calcium intake 1200 mg/day	12 weeks	Patients achieved lower HbA1C levels if they had higher serum 25 OHD levels at 12 weeks	Aljabri KS et al[56]
3	2009	China	LADA patients (n=35)		Randomly assigned Insulin alone (n=18) and insulin plus 1-alpha OH vitamin D_3 (0.25 μg/day) (n=17)	1 year	Plasma C-peptide levels fasting and 2 hours post 75 gm glucose load measured over 6 months. Insulin plus 1-alpha OH vitamin D_3 preserves pancreatic β-cell function in LADA	Li X et al[52]
Negative out comes
4	2010	Rome, Italy IMDIAB XIII trial	Recent onset T1DM with basal C-peptide >0.25 nmol/l (n=34)	11-3; median 18	Randomized double-blind 0.25 μg/day of calcitriol or placebo	2 years	Calcitriol ineffective in protecting β-cell function	Bizzarri C et al[57] IMDIAB XIII trial group
5	2010	Germany	Recently diagnosed T1DM (n=25)	18-39	Calcitriol 0.25 μg/day (n=25). Additional 40 patients randomly received calcitriol 0.25 μg/day or placebo	First group–9 months; second group treatment for 9 months and followed for 18 months	No difference in AUC C-peptide, peak C-peptide and fasting C-peptide between treatment and placebo group	Walter M et al[58]
6	2006	Rome, Italy	Recent onset T1DM (n=70)	13.6 + 7.6	Randomized to calcitriol (0.25 μg on alternate days) or nicotinamide (25 mg/kg daily)	1 year	No significant differences between calcitriol and nicotinamide groups in respect of baseline/ stimulated C-peptide or HbA1c 1 year after diagnosis. But the insulin dose at 3 and 6 months was significantly reduced in the calcitriol group	Pitocco D et al[59] IMDIAB XI trial group

is adequately backed by animal studies and ample epidemiological and observational data in humans. However, no large prospective randomized control studies are available to support a therapeutic role of vitamin D agent in preventing or postponing the disease (Table 1). Hence for now, pragmatism dictates ensuring adequate levels and avoiding deficiency of vitamin D in vulnerable groups as the key priority in day-to-day clinical practice.

Vitamin D in type 2 Diabetes Mellitus
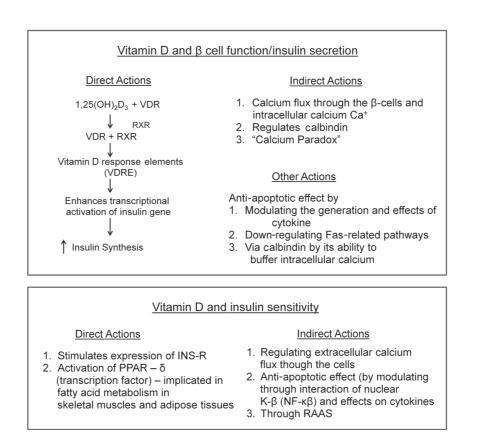
Mechanisms

The main defects that determine the development of T2DM are insulin resistance, pancreatic β-cell dysfunction and systemic inflammation.

β-cell function and insulin secretion

Vitamin D promotes pancreatic β-cell function in numerous ways.

1. Direct actions: Activation of vitamin D occurs in pancreatic β-cells by intracellular 1-α-OHase enzyme. Vitamin D enhances insulin secretion and promotes β-cell survival by modulating the generation and effects of cytokines. The anti-apoptotic action of vitamin D is mediated by down-regulating Fas-related pathways (Fas/Fas-L) (Figure 3).[60,61]

2. Indirect actions: Insulin secretion is a calcium-dependent process and is influenced by calcium flux through the cell membrane by RR.[60] Vitamin D regulates calbindin, a cytosolic calcium-binding protein found in β-cells. It acts as a modulator of depolarization-stimulated insulin release via regulation of intracellular calcium (Figure 3). Thus, vitamin D could indirectly effect insulin secretion additionally by regulating calbindin. Another plausible mechanism could be one whereby low vitamin D status induces secondary hyperparathyroidism (SHPT). The raised parathyroid hormone (PTH) inhibits insulin synthesis

Figure 3. 1,25(OH)$_2$D$_3$ enhances insulin secretion by interacting with the 1,25(OH)$_2$D$_3$-RXR-VDR complex which binds to vitamin D responsive elements (VDRE) found in the insulin gene promoter region, to enhance the transcriptional activation of the insulin gene and increase insulin synthesis.[64] It acts as a modulator of depolarization – stimulated insulin release via regulation of intracellular calcium. INS-R: insulin receptor.

and secretion in β-cells and insulin resistance in target cells by regulating intracellular calcium (Figure 3). The SHPT may actually cause a paradoxical increase in intracellular calcium and in turn may impair the calcium signal needed for glucose induced insulin secretion, this is known as the "calcium paradox".[62]

Vitamin D and insulin sensitivity

In insulin responsive tissues, such as skeletal muscle and adipose tissue, calcium is essential for insulin-mediated intracellular processes. Indeed, a narrow range of intracellular calcium is needed for optimal function.[63] Vitamin D enhances insulin sensitivity by stimulating the expression of insulin receptors and/ or by activating peroxisome proliferator-activated receptor-δ (PPAR- δ).[64] PPAR-δ is implicated in the regulation of fatty acid metabolism in skeletal muscles and adipose tissue (Figure 3). The indirect effect of vitamin D is exerted by regulating calcium flux through the cell membrane and intracellular calcium by RR (Figures 1 and 3). Changes in intracellular calcium in insulin target tissues may contribute to peripheral insulin resistance via impaired signal transduction pathways leading to decreased glucose transporter activity. Vitamin D may promote β-cells survival by inactivation of nuclear factor-ϰB (NF-ϰb) and effects of cytokines.[64] Vitamin D could also affect insulin resistance indirectly through the rennin-angiotensin-aldosterone system (RAAS). Angiotensin II inhibits the action of insulin in vascular and skeletal muscle tissue leading to impaired glucose uptake. Vitamin D suppresses rennin formation and local pancreatic RAAS. Hence, vitamin D could be a negative endocrine regulator of RAAS.[65]

Vitamin D and systemic inflammation

According to the current understanding of the pathogenesis of T2DM, inflammation is postulated to play an important role in insulin resistance, while β-cell function may be affected via cytokine-induced apoptosis. Vitamin D is thought to modulate the expression and activity of cytokines and protect the β-cell against cytokine-induced apoptosis, one such effect being the countering of cytokine-induced Fas expression.[60,61] Other immune modulating effects of vitamin D include blockade of dendritic cell differentiation, inhibition of lymphocyte proliferation, enhanced regulation of T-lymphocytes, development and down-regulation of cytokine expression. These immune modulatory effects of vitamin D might provide additional protection against inflammation-triggered worsening of insulin resistance and, potentially, β-cell function.

Animal studies

Studies in ob/ob mouse showed that $1,25(OH)_2D_3$ treatment improved hyperglycemia, hyperinsulinemia and fat tissue responsiveness to hormones.[69] Vitamin D supplementation had beneficial effects in obese spontaneously hypertensive rats (SHR) and Wistar rats, where there was a reduction in glucose levels in vitamin D supplemented animals.[66] Glucose-mediated insulin secretion from β-cells was impaired in vitamin D deficient rats.[67] Insulin secretion was restored on vitamin D supplementation.[67-69]

Studies in humans

Seasonal variations of glycemic control in patients with T2DM, with a worsening in winter, was explained partly by fluctuations in $25OHD_3$ concentrations on exposure to UV radiation.[44]

1. Cross-sectional studies

Several cross-sectional studies have examined the association between serum $25(OH)D_3$ concentrations and prevalence of T2DM. Most studies have reported an inverse association between $25(OH)D_3$ levels and T2DM,[70] while others failed to show such associations (Table 2).[71] Large population-based studies such as the Third National Health and Nutrition Examination Survey (NHANES III) have disclosed a positive relationship between serum $25(OH)D_3$ levels and insulin sensitivity.[72] In this study, serum $25(OH)D_3$ levels were inversely associated with metabolic syndrome in both genders and all three major racial and ethnic groups (non-Hispanic whites, Mexican Americans and the remaining groups of American adults). Further studies using NHANES III data has confirmed inverse associations between serum $25(OH)D_3$ concentrations and fasting hyperglycemia as well as insulin resistance.[71,72] In a birth cohort study, serum $25(OH)D_3$ concentrations were inversely associated with elevated glycosylated hemoglobin levels.[73]

These observational studies are also supported by laboratory studies. In one study,[74] there was a

Table 2. Clinical trials on vitamin D intervention in Type 2 diabetes mellitus

S. No	Year	Country	Participants (Number of participants)	Age	Vitamin D form and dose intervention	Study duration	Out come	Reference
					Positive out comes			
1	2012	USA	Overweight or obese African-Americans with prediabetes or early diabetes (n=89)		4000 IU/day vitamin D₃	12 weeks	1. Insulin sensitivity decreased by 4% in the vitamin D group (12% increase in the placebo) (p = 0.034). 2. Insulin secretion increased by 12% in the vitamin D group (2% increase in the placebo) (p = 0.024)	Harris SS et al[80]
2	2011	Iran	Type 2 diabetes mellitus (n=72)	30-75	Calcitriol 0.25 μg per day	12 weeks	Insulin secretion (HOMA-%β unchanged in the control group, it increased in the treatment group (p=0.009)	Eftekhari MH et al[81]
3	2011	USA	Adults at high risk of diabetes (n=92)	mean 57	Cholecalciferol 2000 IU/daily *or* calcium carbonate 400 mg twice daily	16 weeks	Disposition index (DI) increased in vitamin D group and decreased in no-vitamin D group (adjusted mean change ± SE: 300 ± 130 compared with -126 ± 127, respectively, P = 0.011) Improvement in insulin secretion (62 ± 39 compared with -36 ±37 mU · L(-1) · min, respectively; P = 0.046)	Mitri J et al[82]
4	2011	Iran	Type 2 diabetes mellitus (n=90)	30-60	3 groups: 1. consume plain yogurt drink (PY - no vitamin D and 150 mg Ca/250 mL); 2. vitamin D-fortified yogurt drink (DY; 500 IU vitamin D₃ and 150 mg Ca/250 mL); 3. vitamin D + calcium-fortified yogurt drink (DCY; 500 IU vitamin D₃ and 250 mg Ca/250 mL)	12 weeks	DY and DCY groups HOMA-IR [-0.6 ± 1.4 (P = 0.001) and -0.6 ± 3.2 (P <0.001)] decreased significantly more than in the PY group	Nikooyeh B et al[83]
5	2010	UK	South Asians with T2DM and vitamin D inadequacy (n=52)	33-72	400 IU of vitamin D₃ and 1200 mg of calcium carbonate/day	16-24 weeks	Reduction in HbA1C levels post treatment	Sabherwal S et al[84]

Table 2. (continue) Clinical trials on vitamin D intervention in Type 2 diabetes mellitus

S. No	Year	Country	Participants (Number of participants)	Age	Vitamin D form and dose intervention	Study duration	Out come	Reference
6	2009	New Zealand	Vitamin D deficient Subjects =42 Placebo =39	41.8 ± 10.1 41.5 ± 9.1	4000 IU Cholecalciferol/day	6 months	Significant improvement in insulin secretion (P<0.003) and insulin resistance (P<0.02) compared to placebo	Von Hurst PR et al[85]
7	2008	USA	Nurses' Health Study (n=83,779)	46	A combined daily intake of >1,200 mg/day calcium and>800 IU vitamin D/day compared with an intake of <600 mg and 400 IU calcium and vitamin D	20 years	33% lower risk of type 2 diabetes with RR of 0.67 (0.49–0.90)	Pittas AG et al[70]
8	2007	USA	Caucasian adults without diabetes (n=314)	> or =65	500 mg calcium citrate and 700 IU vitamin D(3) or placebos daily	3 years	Improved HOMA-IR in subjects with IFG	Pittas AG et al[86]
9	2005	USA	Women's Health Study cohort (10,066 participating in the Women's Health Study analyzed)	>45	Multiple logistic regression models to estimate multivariable odds ratios (ORs) and 95% CIs comparing different dietary intake levels of calcium and vitamin D.	Data analysis	Intakes of calcium and dairy products may be associated with lower prevalence of the metabolic syndrome in middle-aged and older women	Liu S et al[77]
10	2003	Bulgaria	Type 2 diabetes mellitus (n=10)		Treated with cholecalciferol 1332 IU daily for one month	4 weeks	First (FPIS) and second (SPIS) phases of insulin secretion studied by IVGTT. FPIS increased significantly by 34.3%; SPIS by 20.4% (p >0.8 NS). Correlation between the change in FPIS and the change in 25(OH) D level after supplementation (p <0.018)	Borissova AM et al[87]
11	1995	UK	Asians – glucose intolerant (n=22)	44.9	Cholecalciferol 100,000 IU/ stat	8-12 weeks after follow-up	Increase in post OGTT insulin and C-peptide levels	Boucher BJ et al[88]
12	1994	USA	T2DM subjects (n=35)	61	1 µg/day of 1,25 (OH) $_2$ D$_3$ Vs placebo	4 days	Tendency towards better insulin secretion	Orwoll E et al[89]
13	1986	Japan	Type 2 DM subjects (n=14)	54.3	Alphacalcidol 2 µg/day Vs placebo	3 weeks	Improved insulin secretion	Inomata S et al[90]
14	1986	Turkey	Vitamin D deficient women (n=4)	32.7	Cholecalciferol 2000 IU/day oral	6 months	Increased insulinogenic index	Gedik O et al[91]

Table 2. (continue) Clinical trials on vitamin D intervention in Type 2 diabetes mellitus

S. No	Year	Country	Participants (Number of participants)	Age	Vitamin D form and dose intervention	Study duration	Out come	Reference
15	1986	Belgium	Subjects with epilepsy (n=10); elderly subjects (n=15)	Mean 56 and 78 in two groups	25 OH D – loading dose 200 µg and daily 10 µg/day	2 weeks	In epilepsy subjects only - with decrease in fasting insulin and post challenge insulin by OGTT	Nyomba BL et al[92]
					Negative out comes			
1	2010	UK	Type 2 diabetics (n=61)	65	Vitamin D₃ 100,000 IU once orally Vs 200,000 IU once orally	16 weeks	No change in HbA1c, FPG and IR HOMA	Witham MD et al[93]
2	2010	Norway	Non-diabetic overweight/obese individuals (n=438)	38 (21-70)	Vitamin D₃ 40,000 IU/week Vs 20,000 IU/week Vs placebo. All received 500 mg of calcium/day	1 year	No change in HbA1c (P=NS), FPG pr 2hr PG (P=NS), IR HOMA (P=NS) and QUICKI (P=NS)	Jorde R et al[94]
3	2010	USA	Type 2 diabetes mellitus Group-1 (13) Group-2 (11)	61+4 54+3	Subjects randomized to receive 400 IU (Group 1) or 1200 IU (Group 2) cholecalciferol for 4 months	16 weeks	No significant differences were noted in fasting plasma glucose, HbA1c, QUICKI	Patel P et al[95]
4	2010	India	Type 2 diabetes mellitus (n=28)	42-45	300,000 IU of vitamin D₃ administered intramuscularly - vitamin D treated group (group D) or a placebo group (group P)	4 weeks	No significant differences were found between the groups at baseline and four weeks with respect to serum fasting plasma glucose and serum insulin, post OGTT plasma glucose and serum insulin levels, and HOMA-IR	Parekh D et al[96]
5	2009	UK	Recent previous osteoporotic fracture (n=5292)	77	Factorial design to oral 800 IU daily vitamin D₃, 1000 mg calcium (CaCo₃), both, or placebo	24–62 months	No evidence that vitamin D₃ alone or in combination with calcium was able to prevent the development of diabetes	Avenell A et al[97]
6	2009	Norway	Type 2 diabetes mellitus (n=36)	21-75	On metformin and bed-time insulin, were randomized to supplementation with cholecalciferol (40,000 IU per week) versus placebo	6 months	Fasting glucose, insulin, C-peptide, levels not significantly different from baseline	Jorde R et al[98]
7	2009	India	Healthy, middle-aged, centrally obese men (n=100)	> or = 35	Three doses of vitamin D₃ (120,000 IU each; supplemented group) fortnightly or placebo (control group)	6 weeks	No changes in insulin secretion, basal indices of insulin sensitivity with supplementation	Nagpal J et al[99]

Table 2. (continue) Clinical trials on vitamin D intervention in Type 2 diabetes mellitus

S. No	Year	Country	Age	Participants (Number of participants)	Vitamin D form and dose intervention	Study duration	Out come	Reference
8	2009	Germany	48 (18-70)	Healthy individuals (mean 25 OHD levels <12 ng/ml) (n=200)	Placebo Vs vitamin D_3 3332 IU/day	1 year	No change in HbA1C (P=0.90) and FPG (P=0.39)	Zitterman A et al[100]
9	2008	USA	50-79	Healthy postmenopausal women (n=33,951)	Double-blind randomized fashion -1,000 mg elemental calcium plus 400 IU vitamin D_3 daily, or placebo	7 years	Calcium plus vitamin D_3 supplementation did not reduce the risk of developing diabetes over 7 years of follow-up	de Boer IH et al[101]
10	2008	Australia		Adults with vitamin D insufficiency (25OH D levels < or = 50 nmol/L) and without diabetes (12 with impaired glucose tolerance) (n=33)	Two oral doses of 100 000 IU of cholecalciferol, 2 wk apart	4 weeks	No change in blood glucose or insulin mean of 0-120 concentrations, no change in insulin sensitivity (Avignon's insulin sensitivity index) [SiM], QUICKI, HOMA after vitamin D treatment	Tai K[102]
11	2008	UK	64	Stable type 2 DM (n=53)	Vitamin D_2 100,000 IU once (n=17) Vs placebo (n=17)	8 weeks	No change in HbA1C (P=0.74) or IR HOMA (P=0.72) If 25 OHD rise >5 ng/ml IR HOMA significantly improved	Sugden JA et al[103]
12	1997	Germany	26	Healthy males (n=18)	1.5 µg/day of 1,25 $(OH)_2$ D_3 Vs placebo	7 days	No change in insulin sensitivity	Fliser D et al[104]
13	1990	Czechoslovakia	33.4	Ethnicity not reported (n=13)	3 µg/day of 1,25 $(OH)_2$ D_3	4 days	No change in insulin secretion	Zofkova I & Stobla P[105]
14	1989	Denmark	60-63	Men with impaired glucose tolerance (n=14)	Alphacalcidol 2 µg/day	18 months	No change in insulin sensitivity	Lind L et al[106]
15	1987	Sweden	61-65	Caucasian men with IGT and vitamin D sufficient (n=65) Vs	Alphacalcidol 0.75 µg/day Vs placebo	3 months	No change in insulin sensitivity	Ljunghall S et al[107]
16	1984	Denmark	45-54	Postmenopausal women (n=151)	Placebo Vs vitamin D_2 2000 IU/ day, calcium 500 mg/day to all	2 years	No change in FPG (P=NS)	Nilas L, Christiansen C[108]

positive correlation between insulin sensitivity, as measured by hyperglycemic clamp, and $25(OH)D_3$ concentrations even after correcting for confounding factors such as body mass index (r=0.25; P=0.007). There are, however, a number of limitations of many cross-sectional studies. To mention a few, studies investigating the relationship between $25(OH)D_3$ levels and insulin secretion sensitivity have used indirect primary measures, such as fasting insulin and HOMA-R, QUICKI,[75] even though the use of hyperglycemic clamp studies is the gold standard for testing these parameters. In a recent study in postmenopausal women (n=753), $25(OH)D_3$ concentrations were inversely correlated with fasting plasma glucose (r=-0.15; P=0.001).[76]

2. Prospective studies

There are relatively few prospective studies on the association between T2DM and vitamin D status. In the Women's Health Study, a vitamin D intake of 511 IU/day or more was associated with less incident T2DM compared to that observed on 159 IU/day or less.[77] In one of the largest prospective studies, the Nurses' Health Study (USA), women with the highest vitamin D intake, i.e. greater than 800 IU/day, had 33% lower risk of incident diabetes compared to women with intakes of less than 200 IU/day.[78] In the Mini-Finland Health Study, the relative risk of T2DM was 0.6 in subjects with the highest quintiles of $25(OH)D_3$ (70.9 nmol/l) compared to those with the lowest quintiles (22.4 nmol/l; P<0.01). Pooled data from two nested case-control studies by the Finnish Mobile Clinic showed that the men with the highest serum $25(OH)D_3$ quintiles (69.11 nmol/l) *vs* those with the lowest quintiles (22.3 nmol/l; P<0.001) had 82% reduced risk of T2DM incidence.[89]

Role of calcium intake along with vitamin D in T2DM

In the Women's Health Study, calcium intake (after adjustment of vitamin D intake) was inversely associated with prevention of metabolic syndrome.[80] Women with the highest calcium (>1200 mg/day) and vitamin D (>800 IU/day) intake had 33% lower risk of T2DM compared to women with the lowest calcium (<600 mg/day) and vitamin D (<400 IU/day) intake daily.[77]

3. Intervention studies

There are several interventional studies (Table 2) on the effect of vitamin D supplementation on T_2DM and the effect of combined vitamin D and calcium supplementation on T_2DM. It is difficult to draw definitive conclusions from these trials. There are reports of the improvement of insulin secretion six months after supplementation of vitamin D in vitamin D deficient T_2DM subjects.[70] Other studies have reported significant improvement in insulin secretion after variable doses and length of vitamin D supplementation in subjects with T_2DM.[66] Generally speaking, the available studies are limited by a lack of randomized, placebo-controlled dosages and non-reporting of serum 25(OH)D to attain sufficient vitamin D concentrations.

In summary, direct actions of vitamin D promoting β-cell survival and its action on skeletal and adipose tissue that are shown to improve insulin sensitivity are compelling reasons to consider its potential in positively influencing glycemic status in patients with type 2 diabetes. Although an abundance of observational data from the Nurses' Health Study supported the hypothesis of lesser prevalence of hyperglycemia with adequate intake of calcium and vitamin D, equally significant data from a large cohort involving postmenopausal women in the Women's Health Initiative Study in the USA, conducted over a period of 7 years, discredited this positive association. Numerous studies across the globe involving small numbers of patients for shorter durations have yielded similarly conflicting conclusions (Table 2). A recent study has shown that optimizing serum 25(OH)D concentrations and supplementation of calcium in vitamin D deficient individuals improves fasting plasma glucose and pancreatic β-cell reserve (by HOMA model).[109]

Whilst awaiting well-designed, long-term, prospective, randomized intervention trials involving large groups of patients across the globe which will finally resolve the several conflicting issues, the most prudent practice to be undertaken in everyday clinical practice is to ensure adequate vitamin D levels and dietary calcium intake.

CONCLUSIONS

While bearing in mind that vitamin D regulates

both innate and adaptive immunity, thus indicating its potential role in preventing and treating T1DM, an autoimmune disorder of pancreatic islets leading to progressive destruction of insulin secreting β-cells, it is also evident that vitamin D deficiency cannot be the sole determining factor of T1DM. Clearly, both genetic and environmental factors determine the development of the disease. From the existing data, it could be postulated that optimal doses of vitamin D supplementation may influence the pattern of immune regulation and subsequent progression to T1DM in a genetically susceptible individual. However, more studies are required to substantiate the relation between T1DM and vitamin D/vitamin D analogues in the pattern of immune regulations in susceptible individuals.

Multiple studies strongly suggest a role of vitamin D in the wellbeing of β-cells, insulin production and secretion, tissue sensitivity to insulin and the susceptibility to T2DM. An inverse relationship between T2DM and vitamin D is suggested by cross-sectional and prospective studies pointing to a direct link between the risk of T2DM and vitamin D. However, conclusive proof is as yet lacking, this mainly owing to the fact most of the study results have been limited by study design. Thus, large, well designed, controlled, randomized interventional studies of vitamin D and calcium in potential prevention and management of T2DM are urgently required to clarify the relationship between vitamin D and glucose homeostasis in T2DM.

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